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FUNGICIDE RESISTANCE IN POPULATIONS OF ASCOCHYTA RABIEI IN SASKATCHEWAN

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Fungicide resistance in populations of *Ascochyta rabiei* in Saskatchewan

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Abstract

Isolates of *Ascochyta rabiei* collected from chickpea fields in Saskatchewan were assessed for sensitivity to strobilurin fungicides. All of the isolates were sensitive in 2004–2005, but that dropped to 50% in 2006 and 5% in 2007–2008. Isolates from Alberta in 2007–2008 showed the same strong shift to insensitivity to strobilurins. This demonstrated that a shift to insensitivity to pyraclostrobin had occurred in the pathogen population across western Canada, and growers were advised to use other fungicides to manage this disease. A series of experiments were conducted to develop a simple test assessing strobilurin sensitivity in *A. rabiei* for use by the provincial diagnostic laboratory. Based on these studies, assessment of radial growth of single-spore isolates using formulated Headline at 1 $\mu g\ mL^{-1}$ on PDA media is recommended. In field trials to assess the efficacy of alternatives to strobilurins, each of the registered fungicides were highly effective. Assessments of the sensitivity of *A. rabiei* to boscalid, chlorothalonil and mancozeb in Saskatchewan were conducted. Several isolates showed an unexpectedly low sensitivity to chlorothalonil, and additional research is required to assess this reaction more closely. Field assessments of new actives from various companies were conducted, and several showed good efficacy against ascochyta blight. A new fungicide from DuPont (Vertisan) that is very close to registration was examined in detail. Isolates of *A. rabiei* were screened for insensitivity to this active, but no insensitive isolates were found.

Technical Summary

Ascochyta blight, caused by *Ascochyta rabiei*, is the most important factor that limits production of chickpea (*Cicer arietinum*) worldwide. Repeated applications of fungicide are required each year for effective blight management. The baseline sensitivity of the population of *Ascochyta rabiei* to the strobilurin fungicide pyraclostrobin, which had previously provided effective control of ascochyta blight in western Canada, was determined using 106 isolates collected before the registration of pyraclostrobin. The mean EC_{50} value was about 1 $\mu g\ mL^{-1}$. Isolates collected from random chickpea fields in Saskatchewan from 2004–2007 were assessed for sensitivity to pyraclostrobin at 1 $\mu g\ mL^{-1}$; all isolates were sensitive in 2004–2005, 50% in 2006, and only 3% (at 5 $\mu g\ mL^{-1}$) in 2007. A similar pattern of response was noted for azoxystrobin (trade name Quadris), which demonstrates that there is strong cross-resistance between these two strobilurin fungicides.

The project is being conducted in close collaboration with researchers in Alberta. This collaboration provides us with an opportunity to confirm trends based on collections of material from chickpea production areas in both provinces. Only 1 of 122 isolates collected in Alberta in 2007 was sensitive to 1 $\mu g\ mL^{-1}$ of pyraclostrobin. There was a 90x increase in the mean EC_{50} of Alberta isolates collected in 2007 compared to the baseline isolates. This demonstrates a shift to insensitivity to pyraclostrobin in the pathogen population across western Canada, which corresponds to a similar shift that occurred in the north-central USA at the same time (Wise et al. 2008).

Studies were conducted to develop a screening protocol for use by the provincial diagnostic laboratory in Saskatchewan to differentiate sensitive and insensitive isolates.

Formulated active provided the same response as the technical grade product, SHAM did not have an important influence on assessments, and mass transfer isolates generally provided a similar pattern of response as single-spore isolates. Similarly, conidial germination and radial growth assays showed the same pattern of response, even though EC_{50} values differed substantially between the two assessments. Assessment of radial growth of single-spore isolates using formulated Headline at 1 $\mu g\ mL^{-1}$ on PDA media is recommended. Studies of a small number of sensitive and insensitive isolates indicate that the insensitive isolates are as pathogenic as sensitive isolates, and so this characteristic will likely persist in the pathogen population.

In field trials to assess the efficacy of alternatives to strobilurins, each of the registered fungicides was highly effective. The order of fungicide application in a season was much less important than applying the fungicide at the right time to limit epidemic increase. Assessments of the sensitivity of *A. rabiei* to boscalid, chlorothalonil and mancozeb in Saskatchewan were conducted. Several isolates showed an unexpectedly low sensitivity to chlorothalonil, and additional research is required to assess this reaction in more detail. Field assessments of new actives from various companies were conducted, and several showed good efficacy against ascochyta blight. A new fungicide from DuPont (Vertisan) that is very close to registration was examined in detail. The baseline sensitivity of *A. rabiei* to this active was quantified and isolates were screened for insensitivity, but no insensitive isolates were identified. Additional studies of cross-resistance with boscalid (same mode of action) are required.

A graduate student (Nael Thaher) was recruited to work on the project starting in September 2008. He completed his course work in the spring of 2009 and successfully defended his thesis at the University of Guelph in January 2011.

Technical Report

BACKGROUND

Ascochyta blight, caused by *Ascochyta rabiei* (Pass.) Labr. (teleomorph, *Didymella rabiei* (Kovacheski) Arx.), can cause 100% yield loss in chickpea (*Cicer arietinum* L.), and is the most important constraint to chickpea production wherever chickpea is grown (Chongo and Gossen 2003). Management of ascochyta blight on chickpea in western Canada is largely dependent on repeated application of foliar fungicides throughout the season, because cultivars with strong resistance to the pathogen are not available (Ahmed et al. 2006, Vail and Banniza 2008). Many of the fungicides registered for control of ascochyta blight on chickpea in Canada belong to single site-specific mode of action groups. These include the strobilurins, which are quinone outside inhibitors (QoI), and boscalid, which is a succinate dehydrogenase inhibitor (SDHI- pyridine carboxamide). In addition, fungicides that are active at multi-sites, such as chlorothalonil and mancozeb, still form a substantial proportion of the usage pattern, used either singly in rotation with site-specific fungicides, or in tank mixtures.

When this project was initiated, the most effective fungicides against ascochyta blight were the strobilurins. When strobilurin fungicides (FRAC group 11) became available for use against ascochyta blight in the mid 2000s, they immediately became the dominant fungicides and provided high levels of control. Unfortunately, *A. rabiei* has been identified as being at high risk of developing resistance to these fungicides, and resistance to strobilurin fungicides was present in the pathogen population in Saskatchewan even before these active ingredients were released. Extensive use of QoIs resulted in a rapid and widespread insensitivity in *A. rabiei* populations in the Northern Great Plains region of North America (Chang et al. 2007; Wise et al. 2009). A widespread breakdown in fungicide efficacy that occurs without warning or the availability of adequate alternatives could devastate this industry, which is just recovering from a breakdown in resistance in the dominant cultivar (Sanford) that causes the chickpea acreage in Saskatchewan to decline from over 500,000 ha in 2001 to around 100,000 ha in 2004. Saskatchewan chickpea producers need to know if there is insensitivity to fungicides in the pathogen population, and about its importance, prevalence, and distribution.

Pyraclostrobin, a strobilurin fungicide has been the most commonly used fungicide in western Canada since it's registration for this use in 2003. Pyraclostrobin is locally systemic and has both protective and preventive modes of action (inhibits spore germination and early infection) (Butzen et al. 2005). It is classified as a quinone outside inhibitor (QoI) and blocks electron transport at the quinol-oxidizing site of the cytochrome bc1 complex (complex III) in the mitochondrial respiratory pathway (Bartlett et al. 2002; Grasso et al. 2006). The site-specific mode of action of this active increases the potential for resistance to develop in fungal populations. In fact, several fungal pathogens are reported to have reduced levels of sensitivity to QoI fungicides due to single amino acid substitutions in the cytochrome b site (Chin et al. 2001; Grasso et al. 2006; Kim et al. 2003; Ma et al. 2003; Pasche et al. 2005).

In western Canada, resistance to strobilurin fungicides in *A. rabiei* was first reported in 2004 from isolates collected before exposure to strobilurins (Gossen and Anderson

2004). Similarly, variation in sensitivity to pyraclostrobin was reported in four isolates of *A. rabiei* collected from southern Alberta in 2003–2004 (Chang et al. 2007). Since then insensitivity has also been reported in the Northern Great Plains of the USA (Wise et al. 2008, 2009). The Fungicide Resistance Action Committee (FRAC 2005) recommends the use of 50 isolates of the pathogen that have never been exposed to the candidate fungicide to determine the baseline EC_{50} value.

In the presence of respiration inhibiting fungicides like pyraclostrobin, some fungi are able to use an alternative respiratory pathway to bypass complex III and IV in the mitochondrial respiration chain, allowing fungal spores to germinate and mycelium to grow even in the presence of high fungicide concentrations (Olaya and Köller 1999; Vincelli and Dixon 2002; Ziogas et al. 1997). Salicylhydroxamic acid (SHAM) is often used in fungicide testing to prevent fungal pathogens from using the alternative respiratory mechanism (Olaya and Köller 1999), and may be important because *A. rabiei* has been shown to use this pathway (Wise et al. 2008).

When the current study was initiated, levels of insensitivity to the most effective fungicides available (QoIs) were increasing rapidly in populations of *A. rabiei* on the Northern Great Plains, and there was a need to develop a simple and effective screening test that could be used by diagnostic laboratories to assess insensitivity of strobilurin fungicides in *A. rabiei*. To address this need, a study comparing radial growth and conidial germination assessments, with and without SHAM, was undertaken as the first step towards developing a consistent method of assessment of the incidence of insensitivity in *A. rabiei*. Similarly, assessment is usually conducted using single-spore isolates to ensure a consistent response in the test, and technical grade active ingredient is used to ensure that the formulation of the commercial product does not confound the results. However, the requirements to develop single-spore colonies increases the time required for testing, and technical grade active ingredient is not readily available or easy to obtain. Thus, the importance and impact of each of these components in assessment of insensitivity in *A. rabiei* was of interest in this study.

Fitness is defined as the expected contribution of individuals to the consequent generations through survival and multiplication. In the presence of selection pressure, insensitive strains show higher fitness than sensitive ones, whereas in the absence of selection pressure, insensitive strains may have impaired survival. Fitness costs associated with genes for insensitivity to a particular fungicide may affect the development and evolution of fungicide insensitivity in pathogen populations. Variation in fitness costs can be associated with different genes for insensitivity or differences in genetic backgrounds (Peever & Milgroom, 1995). The fitness of insensitive isolates is very useful in predicting the build-up of an insensitive pathogen population and estimating the risk of insensitivity for a new fungicide. For example, fungicide-insensitive isolates in some pathogens are less fit, less able to infect and reproduce, or have impaired survival in the absence of the fungicide. If there is a reduction in fitness associated with insensitivity to fungicides then the frequency of insensitive isolates in the pathogen population declines over time as a result of competition with sensitive isolates. The fitness costs associated with QoI insensitivity in the absence of a fungicide in *A. rabiei* are not known.

Penthiopyrad (trade name Vertisan) is a new succinate dehydrogenase inhibitor (SDHI, FRAC group 7) fungicide that is being evaluated for use on chickpea for control of ascochyta blight, but is not yet in commercial use in Canada. Its mode of action is similar to carboxamide fungicides such as boscalid. Penthiopyrad is a broad-spectrum fungicide with preventive, curative, and residual activity with uptake into the plant. Evaluation of the impact of penthiopyrad on blight severity and seed yield of chickpea in western Canada has indicated that penthiopyrad reduces the severity of blight and increases seed yield (see previous reports in this section). The results of these field trials on the efficacy of penthiopyrad indicated that two applications of penthiopyrad on a highly susceptible chickpea cultivar provided high levels of control of ascochyta blight.

Boscalid is a relatively new SDHI fungicide (FRAC group 7). It is a systemic fungicide with protectant and curative properties that was registered for use on chickpea in Canada in 2004. The site-specific mode of action and the widespread use of boscalid on chickpea may increase the risk of developing insensitivity in the pathogen population (Wise et al. 2008). As a result, there is a need to monitor sensitivity to boscalid and to assess the potential for cross-resistance between these two actives in *A. rabiei*. Cross-resistance between the SDHIs and other classes of fungicides such as strobilurins, triazoles, anilinopyrimidines, and dicarboximides has not been detected.

The risk of insensitivity to SDHI fungicides such as boscalid and penthiopyrad is medium (www.frac.info), but insensitivity to boscalid has already been reported in a number of fungal pathogens, and cross-resistance between penthiopyrad and boscalid was recently reported in *Didymella bryoniae* (Thomas et al. 2010). To date, insensitivity to boscalid in *A. rabiei* has not been reported. The baseline sensitivity to boscalid (EC_{50} values) in *A. rabiei* isolates based on conidial germination assay ranged from 0.02 to 0.5 µg/mL, with a mean of 0.19 µg/mL (Wise et al. 2008). This distribution had a broad range of EC_{50} values, which may indicate that *A. rabiei* has the potential for developing insensitivity to boscalid.

Response of a fungal population to development of insensitivity is often either qualitative (monogenic) or quantitative (polygenic). In qualitative insensitivity, a single or major gene causes a high level of insensitivity to a fungicide that result in a complete loss of control in the pathogen populations. The frequency level of sensitive isolates will decrease and the insensitive isolates will increase rapidly in response to selection and leading to qualitative population response because the selection from the fungicide. This leads to a qualitative population response because the insensitive population is not affected by the fungicide, so selection proceeds rapidly. This type of response is called disruptive selection, and frequently occurs for fungicides such as the strobilurins. In contrast, the response in quantitative insensitivity is mediated by several interacting genes and the decline in disease control and sensitivity of pathogen populations occurs gradually. This response is called directional selection. This type of response frequently occurs for the SDHI fungicides.

Fungicides with multi-site activity, such as mancozeb and chlorothalonil are generally non-systemic and have only protectant activity. They create a barrier on the plant surface that prevents the development of pathogen and the process of infection. The biochemical action of these fungicides is not specific and involves many steps in pathogen metabolism, which in turn leads to a general disruption of metabolism and cell

integrity. Despite, the widespread use of these fungicides, they have remained efficacious. However, an *in vitro* study noted high levels of variation in the sensitivity of a small number of isolates of *A. rabiei* to the multi-site inhibitor fungicides chlorothalonil and mancozeb in western Canada and interpreted this wide range as an indication of a decline in sensitivity (Chang et al. 2007). Another study reported that chlorothalonil inhibited conidial germination of *A. rabiei* at low concentrations *in vitro* but did not inhibit mycelial growth, even at high concentrations (Demirci et al. 2003). Given the low risk of loss of sensitivity to multi-site inhibitor fungicides, loss of sensitivity in *A. rabiei* appears to be unlikely, but insensitivity could have an important impact on blight management in North America.

The protectant fungicides chlorothalonil and mancozeb are used extensively to manage a broad range of pathogens on many hosts. They are effective mixing partners for other fungicides. Growers in western Canada use chlorothalonil and mancozeb as preventative applications prior to flowering in chickpea and alternate or tank-mix them with systemic fungicides for control of ascochyta blight on chickpea.

OBJECTIVES

The objectives of the study are to: i) collect isolates of *Ascochyta rabiei* from chickpea to represent the pathogen population in Saskatchewan, ii) assess the frequency and distribution of strobilurin fungicide insensitivity in these isolates, iii) assess registered protectant fungicides to ensure that they are still efficacious, and iv) develop baseline values of efficacy for the new classes of fungicides that have been registered for management of ascochyta blight on chickpea.

The widespread occurrence of insensitivity to strobilurins was confirmed early in the project, so the focus of the project shifted to a detailed examination of factors affecting assessment of this insensitivity and examination of fungicide alternatives for management of ascochyta blight.

PROJECT ACTIVITIES AND ACHIEVEMENTS

A. SENSITIVITY TO STROBILURINS OF *A. RABIEI* IN SASKATCHEWAN

MATERIALS AND METHODS

Baseline: A preliminary study was conducted prior to the initiation of the research agreement to assess the reaction of 106 isolates of *A. rabiei* (88 isolates collected in Saskatchewan, with the remainder from international sources) to two strobilurin fungicides, Quadris (azoxystrobin, Syngenta Crop Protection Canada, Inc., Guelph, ON) and Headline (pyraclostrobin BASF Canada Inc., Toronto, ON). All of these mass-transfer isolates were collected before strobilurin fungicides were registered for use on chickpea in western Canada. The assessments were based on inhibition of mycelial growth on commercial potato dextrose agar at 0, 1, 10, 100 and 1000 $\mu g\ mL^{-1}$ of active ingredient, using the formulated fungicide as the source of the active. The fungicide was added just prior to pouring the agar into Petri dishes. For each fungicide concentration,

two plates per isolate were inoculated (mycelium-side down) with 6-mm agar blocks cut from the margin of an actively growing culture. Dishes of unamended agar were used as controls. The dishes were incubated under continuous fluorescent light and 20 ± 2° C. A 2-wk incubation period was used to allow the control colonies to grow across at least half of the diameter of each plate. The mean of the measurement of the colony at its widest point and a perpendicular measurement of the same colony was used to determine radial growth of the each colony. Growth measurements were converted to a percentage of radial growth on unamended media.

To provide a more precise estimate of the baseline reaction as part of the current study, 42 of the Saskatchewan isolates from the preliminary trial were grown on chickpea agar (1.5% ground chickpea seed, 1.5% agar) amended with 0, 0.2, 1, 2, 3, and 4 μg mL^{-1} of pyraclostrobin as formulated product. For each fungicide concentration, there were three plates per isolate, and the dishes were incubated under fluorescent light at 16/8 hr light/dark period. The trial was repeated.

A linear regression of the mean percent growth of the isolate versus the natural log of fungicide concentration was obtained, and the effective concentration to inhibit growth by 50% (EC_{50}) was calculated for each isolate.

Assessment of sensitivity of *A. rabiei* in Saskatchewan: Prior to the initiation of the study, disease surveys of chickpea fields in the main production areas of Saskatchewan were conducted from 2004–2006. In each field, chickpea plants were collected at each of two sites, and (about) five mass transfer isolates of *A. rabiei* were made from lesions collected at each site. In 2006, isolates were assessed from seven fields where the grower thought that there had been a breakdown in resistance, and compared with isolates from random fields in the region. Each isolate was stored as conidia at -70° C until they were required for testing. The radial growth of each isolate was assessed as described previously on PDA amended with 1 μg mL^{-1} of azoxystrobin (as Quadris) and pyraclostrobin (as Headline), with two dishes per isolate and one unamended control. The percent reduction of radial growth due to fungicide was calculated, and isolates were classified as insensitive (<30%), intermediate (30–59%), or sensitive (≥ 60% reduction).

In 2007, a larger number of fields were assessed (Fig. 1) and field histories (focussed on recent cropping and fungicide application history) were obtained wherever possible. Azoxystrobin was dropped from the study, and the concentration of pyraclostrobin for the assessment was increased to 5 μg mL^{-1} in an attempt to minimize the number of isolates with an intermediate sensitivity to strobilurin fungicides.

In 2008, a small survey was conducted and 81 isolates were collected from fields across southern Saskatchewan. Azoxystrobin was dropped from the study, and the concentration of pyraclostrobin for the assessment was held at 5 μg mL^{-1} in an attempt to minimize the number of isolates with an intermediate sensitivity to strobilurin fungicides. The radial growth of 81 mass-transfer isolates of *A. rabiei,* collected from commercial chickpea fields in Saskatchewan in 2008, was assessed using PDA amended with formulated pyraclostrobin (Headline) at 5 μg mL^{-1} a.i. plus SHAM at 100 μg mL^{-1}. The radial growth of isolates was evaluated as described previously. The control treatment was amended with SHAM. Growth inhibition for each isolate was determined as

described previously. Reduction in growth of < 40%, 40–70%, and > 70% was used to differentiate insensitive, intermediate and sensitive isolates, respectively.

In 2009–2010, small surveys were conducted to assess the impact of the loss of strobilurins on management of ascochyta blight, and to collect isolates for future assessment of fungicide sensitivity.

Assessment of sensitivity of *A. rabiei* in Alberta: To assess the sensitivity to pyraclostrobin in field populations of *A. rabiei* in Alberta, isolates were collected from infected chickpea plants in southern Alberta in 2007 (Fig. 1A) and were purified to single-spore isolates. The isolates were grown on chickpea-agar medium and spores were harvested in sterile water, centrifuged to form a pellet and transferred into a 1.5 mL Eppendorf tube. A total of 122 isolates were stored at –20°C until they were required for testing.

The isolates were grown on chickpea agar medium and then transferred as described above onto chickpea-agar plates amended with pyraclostrobin at 1 $\mu g\ mL^{-1}$, which was the mean EC_{50} value of the baseline isolates. Each isolate was inoculated onto three fungicide-amended Petri dishes, with three unamended dishes as controls. After inoculation the plates were placed in an incubator in a completely randomized design. The radial growth of each isolate was measured as previously described, the percent reduction of radial growth due to fungicide was calculated, and the frequency distribution of sensitivity was determined. The experiment was repeated.

Pathogenic fitness: Seed of the kabuli cv. CDC Xena (highly susceptible to ascochyta blight) was sown in 23-cm diameter plastic pots filled with soil-less mix (MIX LA4, Sun Gro Horticulture Canada Ltd., Seba Beach, AB) and thinned to three plants per pot. The seedlings were maintained in the greenhouse at about 20°:16° C (day: night) temperature, with a 16-h photoperiod and 200-250 $\mu E\ m^{-2}\ s^{-1}$ light intensity from high-pressure sodium and incandescent bulbs, and watered as needed.

Four pyraclostrobin-sensitive (FLD3A/7, FLD3A/8, FLD3A/10, FLD8/6) and four pyraclostrobin-insensitive (FLD6/1, FLD9/8, FLD2/2, FLD2/8) single-spore isolates of *A. rabiei* collected in 2008 in Saskatchewan were selected for this study based on response to a discriminatory dose of 5 µg/mL pyraclostrobin as described previously. Conidial suspensions of 2×10^5 conidia/ mL were prepared from 14-day-old cultures of each isolate on half-strength commercial oatmeal agar. The plants were inoculated 3 weeks after seeding by spraying to runoff with the spore suspension using a hand-pump sprayer. Following inoculation, plants were placed in a clear plastic mist chamber for 48 h at high RH to maintain high humidity and promote infection (Chongo and Gossen 2001). Non-inoculated pots of plants sprayed with sterile distilled water were included as a control. There were five replicates (pots) per isolate, arranged in a greenhouse in a randomized complete block design.

Each plant was visually assessed each day to determine the time interval from inoculation until initiation of visible lesions and initiation of pycnidia. Blight severity was rated on leaves, stems, and whole plants at 14 days after inoculation using the rating scale that assesses both quantitative and qualitative disease reaction. Each plant was rated

on a 0–9 scale where 0 = no symptoms; 1 = few very small (<2 mm^2) lesions on leaves and stems, <2% plant area affected (PAA); 2 = very small (<2 mm^2) lesions, 2-5 % PAA; 3 = many small lesions (<2 to 5 mm^2), 5 to 10% PAA; 4 = many small lesions, few large (>5 mm^2) lesions, 10 to 25% PAA; 5 = many large lesions, 25 to 50% PAA; 6 = lesions coalescing, 50 to 75% PAA, 7 = lesions coalescing with stem girdling, 75 to 90% PAA; 8 = stem girdling or breakage, >90% PAA; 9 = plants dead (Chongo et al. 2004).

Data analysis: All of the statistical analyses were conducted using SAS 9.1 (SAS Institute, Cary, NC), and analysis of variance was conducted using PROC MIXED. Differences are significant at $P = 0.05$ unless otherwise stated. The repetitions of each experiment did not differ, so the data were pooled for subsequent analyses. The only exception was the study on isolate fitness, where the repetitions of each experiment were different. Where appropriate, the reduction of radial growth or conidial germination (%) for each isolate in each replicate in relation to the $\log_{10}$ of the fungicide concentration was used in PROC PROBIT to calculate the EC_{50} values (the concentration required to inhibit 50% of radial growth or conidial germination). In the study to assess pathogenic fitness of pyraclostrobin-sensitive and-insensitive isolates, blight severity and lesion counts were subjected to mixed model analysis of variance. Isolate category (sensitive vs. insensitive) was a fixed effect, and isolate nested within category, replication and repetition were random effects. The distribution of disease severity rating and lesion counts of each isolate was tested for normality using the Shapiro-Wilk test (PROC UNIVARIATE).

RESULTS

Baseline: In a trial to assess the sensitivity of *A. rabiei* to strobilurin fungicides, 1 of the 106 isolates was insensitive to both azoxystrobin and pyraclostrobin, with little growth inhibition even at 1000 μg mL^{-1}. Several isolates were rate insensitive, with linear growth constant from 1 to 1000 μg mL^{-1}. The majority of the isolates were highly sensitive to both fungicides and exhibited little or no growth at 1 μg mL^{-1}. The insensitive isolate had been collected in southern Saskatchewan. In the detailed assessment of baseline isolates, the mean EC_{50} value for linear growth of the 42 baseline isolates was 1 μg mL^{-1} of pyraclostrobin. Pyraclostrobin consistently had a stronger impact on growth of *A. rabiei* than azoxystrobin at a given concentration, but the same pattern of response.

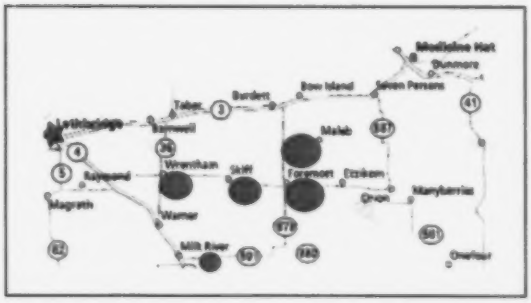
Saskatchewan: All 53 of the isolates collected in Saskatchewan from 2004–2005 were sensitive to pyraclostrobin at 1 μg a.i. mL^{-1} and 49 isolates were sensitive to azoxystrobin (4 were assessed as intermediate, with 30–59% reduction in linear growth). However, the frequency of sensitive isolates collected from random fields in 2006 was much lower: for pyraclostrobin, 17 of 37 isolates were sensitive, 17 were intermediate and 3 were insensitive, and for azoxystrobin, 14 of 37 isolates were sensitive; 2 were intermediate and 21 were insensitive. In fields where a breakdown in efficacy was suspected, the shift was even more dramatic. In six of seven fields, none of the 57 isolates were sensitive to either active: 2 isolates were intermediate to azoxystrobin and 55 were insensitive; 19

were intermediate to pyraclostrobin and 38 were insensitive. In the remaining field, all seven isolates were sensitive to both fungicides.

In 2007, isolates were assessed on pyraclostrobin at 5 μg mL^{-1}; 4 of 143 isolates were sensitive to pyraclostrobin, 135 were intermediate and 4 were insensitive. In 2008, 7 of 81 were sensitive, 27 were intermediate, and 47were insensitive.

Alberta: Only 1 of 122 isolates was sensitive to pyraclostrobin at the baseline EC_{50} value. Therefore, the EC_{50} values of nine isolates collected from Alberta in 2007 were determined as described previously using 0, 1, 5, 10, 20, 40, 80, 100 and 150 μg mL^{-1} of pyraclostrobin. The mean EC_{50} value of these isolates was used to test the sensitivity of the isolates from the 2007 collection. The EC_{50} of the isolates collected from Alberta in 2007 was 90 fold higher than the baseline sensitivity level, ranging between 78 and 102 μg mL^{-1} (data not shown). The distribution of EC_{50} values for the baseline isolates was heavily skewed to the right of the median value (Fig. 2A). This degree of skew supports the initial observation that a few isolates were able to grow slowly at fungicide concentrations much higher than the majority of isolates. In contrast, the sensitivity of 122 Alberta isolates at 90 μg mL^{-1} of pyraclostrobin (Fig. 2B) was normally distributed. In fact, the radial growth of 40 isolates on this high concentration of fungicide was faster than on media without fungicide.

A



B

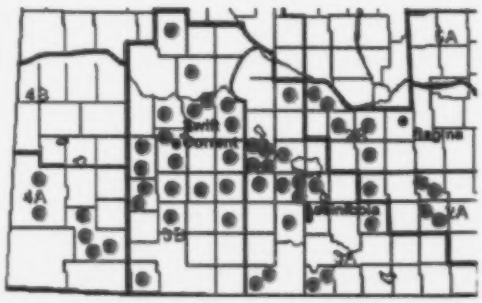


Fig. 1. Collection sites of isolates of *Ascochyta rabiei* from Alberta (A) and Saskatchewan (B) in 2007.

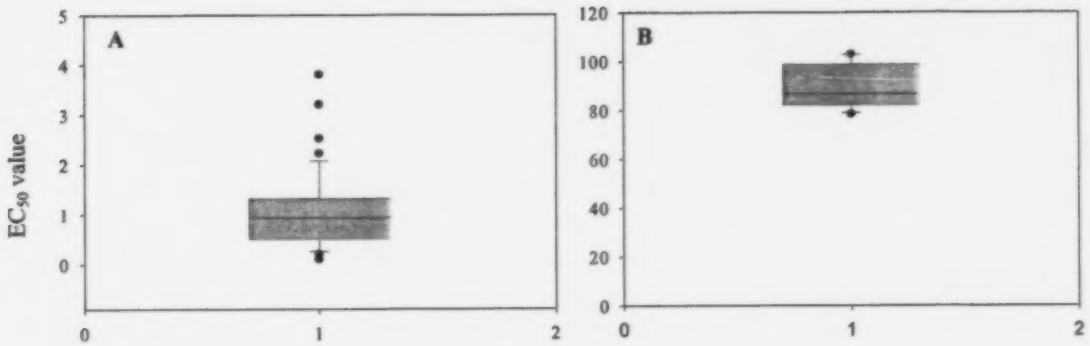


Fig. 2. Distribution of EC_{50} values (µg/mL) of baseline isolates of *Ascochyta rabiei* from Saskatchewan (A) versus isolates from Alberta collected in 2007 (B).

Fitness of sensitive and insensitive isolates: The pathogenic fitness of each of eight isolates (four sensitive and four insensitive) was determined by measuring their ability to cause disease on susceptible chickpea plants. There were no differences between sensitive and insensitive isolates in the absence of fungicide for blight severity, based on first appearance of lesions or pycnidia in the three repetitions of the study (Fig. 3) or for numbers of lesion in the two runs where counts were made (Fig. 4). Lesions were produced 4 days after inoculation and pycnidia were produced 6–7 days after inoculation for each isolate.

The blight severity ratings were normally distributed for the three runs ($P = 0.69$, 0.32, 0.13), while lesion counts did not have a normal distribution ($P = 0.002$) in the first run, but were normally distributed in the second run ($P = 0.72$). Blight severity differed among the repetitions, and number of lesions differed between the two repetitions where it was assessed.

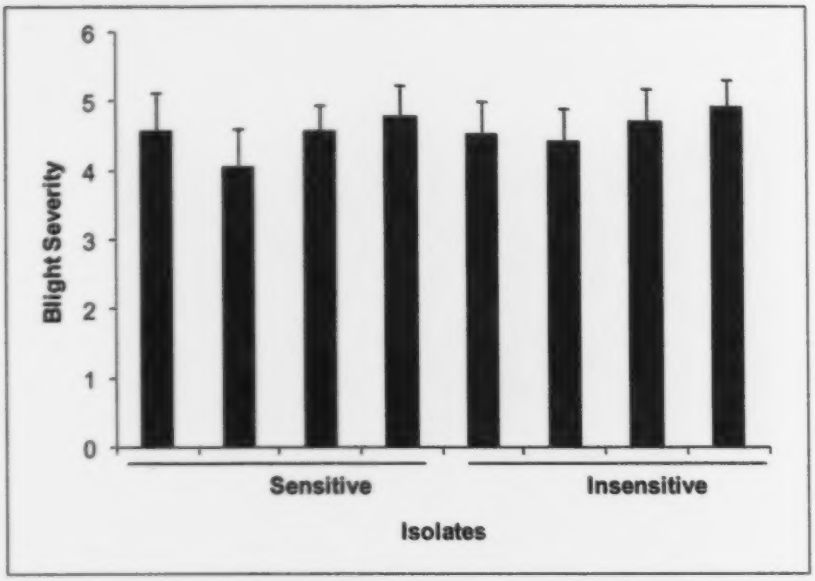


Fig. 3. Mean ascochyta blight severity on chickpea inoculated with spore suspensions of pyraclostrobin-sensitive and -insensitive isolates of *Ascochyta rabiei,* combined across repetitions of a study under controlled conditions. Capped lines represent standard error (n = 15).

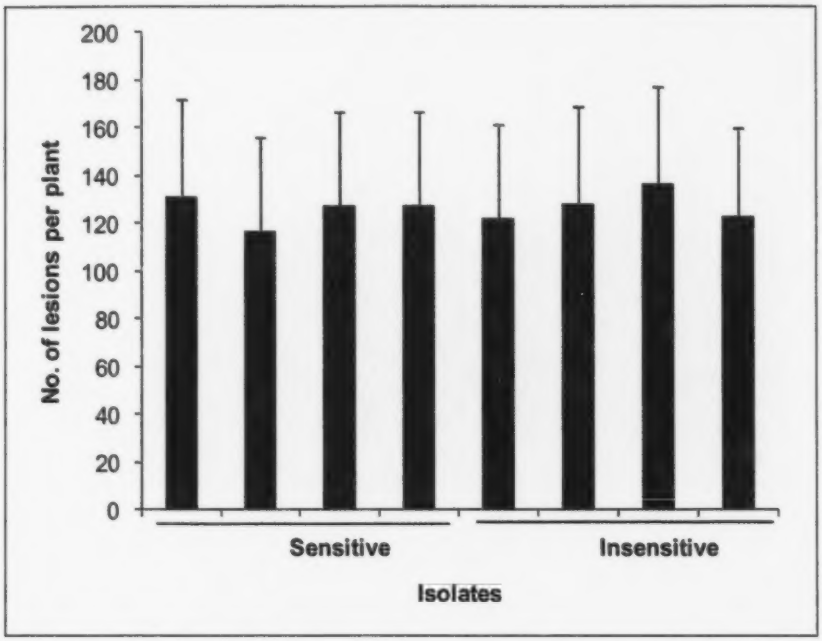


Fig. 4. Mean number of ascochyta blight lesion per plant on chickpea plants inoculated with spore suspensions of pyraclostrobin-sensitive and -insensitive isolates of *Ascochyta rabiei* combined across repetitions of a study under controlled conditions. Capped lines represent standard error (n = 10).

DISCUSSION

The mean EC_{50} for pyraclostrobin in isolates of *A. rabiei* collected in the USA before 2002 was 0.002 μg mL^{-1} (Wise et al. 2008). Similarly, the mean EC_{50} of four isolates of *A. rabiei* collected in 2003–2004 in Alberta was 0.01 μg mL^{-1} (Chang et al. 2007). In the current study, the mean EC_{50} of the baseline isolates was 1.0 μg mL^{-1} (range 0.3–3.8), which is over 100x higher than the previous baselines. However, there was a difference in the test procedure to evaluate the EC_{50} values. Both of the earlier studies used conidial germination for assessment, but radial growth was assessed in the current study. The mean EC_{50} for conidial germination of the isolates collected from Alberta in 2007 was 1.9 μg mL^{-1}, which is 190x higher than those collected in 2003–2004.

The isolates collected from random fields in Saskatchewan from 2004 to 2007 show a clear pattern of increase in the frequency of insensitivity to strobilurin fungicides over time. In 2004–2005, 100% of the isolates assessed were sensitive to pyraclostrobin. In 2006, 50% were sensitive, 3% in 2007, and 9% were sensitive in 2008. Moreover, in 6 of 7 fields where a breakdown in fungicide efficacy was suspected in 2006, none of the isolates were sensitive. We can infer from this observation that reduction in sensitivity results in a breakdown in disease control.

A similar pattern was observed in Alberta. Isolates collected in 2007 were highly insensitive to pyraclostrobin compared to collections made in 2003–2004 (Chang et al.

2007), where most isolates were sensitive to 0.25 μg a.i. mL^{-1} in assessment of radial growth. The mean EC_{50} for conidial germination of the isolates collected from Alberta in 2007 was 1.9 μg a.i. mL^{-1}, compared to 0.01 μg mL^{-1} for isolates collected in 2003–2004 (Chang et al., 2007) and 0.002 μg mL^{-1} for isolates collected in the USA before 2002 (Wise et al., 2008).

Since 2000, the population of *A. rabiei* in western Canada has become more aggressive on cultivars that had previously been partially resistant. This shift has left producers even more reliant than previously on application of foliar fungicides for management of ascochyta blight. After the introduction of strobilurins in 2003, a QoI fungicide was applied to a high proportion of chickpea fields in the region each year from 2004 to 2006, so isolates collected in 2007 and 2008 are derived from populations that had been exposed repeatedly to this class of fungicides. The current study demonstrates that the pathogen population is now generally insensitive to pyraclostrobin. Also, the isolates showed a similar reaction to azoxystrobin from each location and year assessed. As a result, the information on location and cropping history that was collected together with the samples in 2007 was not used further, because such a large proportion of the pathogen population in every field was insensitive to QoIs. Instead, a substantial effort was made to inform both industry and growers that QoIs were no longer effective against this pathogen.

Insensitivity to QoI fungicides is often qualitative, resulting in cross resistance with all of the fungicides with the same mode of action. Therefore, we anticipate that the population is insensitive to all QoI fungicides. This shift has increased the risk of damaging blight epidemics for chickpea producers of Western Canada. The rapid distribution and simultaneous shift to insensitivity observed simultaneously across the Northern Great Plains region of Canada and the USA (Wise et al. 2008, 2009) may indicate that gene transfer occurs across the region via long-distance dispersion of air-borne ascospores (Gossen *et al.*, 2009).

There were no differences in the relative fitness of four sensitive and four insensitive isolates based on assessments of days to the first appearance of lesions and pycnidia, lesion numbers or blight severity. There appears to be little or no fitness penalty associated with QoI insensitivity in *A. rabiei* in the absence of selection pressure from the fungicide, This observation is supported by the that pyraclostrobin-insensitive isolates were initially found in a population where pyraclostrobin had never been used (Gossen & Anderson, 2004). Since there appears to be no fitness costs associated with insensitivity, discontinuance of QoI fungicides is unlikely to result in a rapid decline in the frequency of insensitive isolates in the pathogen populations and QoI-insensitive isolates will likely persist. Managing the risk of the development of fungicide insensitivity in *A. rabiei* by rotating or mixing fungicides with different modes of action is required to avoid the selection of populations of *A. rabiei* that are insensitive to other classes of fungicides. However, it is important to note that this observation is based on a limited number of isolates. Assessment of a larger number of QoI-insensitive and-sensitive isolates is strongly recommended.

B METHODOLOGY FOR TESTING SENSITIVITY

MATERIALS AND METHODS

Selection of isolates: Three sensitive, four intermediate, and three insensitive isolates from the isolates collected in Saskatchewan in 2007 were selected at random for use in the study based on the percent reduction of radial growth compared to the nontreated check. The levels of reduction in growth chosen to differentiate insensitive, intermediate and sensitive isolates were < 40%, 40–70%, and > 70%, respectively.

Assessment of radial growth and conidial germination, with and without SHAM: Fungicide sensitivity was evaluated based on radial growth and conidial germination. Each study was conducted on PDA amended with pyraclostrobin in a formulated product (Headline 250 EC; BASF Canada Inc., Mississauga, ON) with and without the addition of salicylhydroxamic acid (SHAM, 99%; Sigma-Aldrich, MO, USA). A stock solution of Headline was prepared at a concentration of 100 mg pyraclostrobin per mL in water. Autoclaved PDA was amended with Headline to achieve final concentrations of 0, 0.1, 1, 10, 100, and 1000 µg pyraclostrobin per mL for radial growth assessments, and 0, 0.0001, 0.001, 0.01, 0.1, 1, and 10 µg pyraclostrobin per mL for conidial germination assessments. SHAM was dissolved in methanol at 100 µg mL^{-1}. The fungicide and SHAM were added just prior to pouring the media into Petri dishes. Control treatments were amended with SHAM for comparison with treatments where SHAM was added to the media and a control. Each study was arranged as factorial completely randomized design with one dish per replicate and three replicates per treatment. The experiment was performed twice. The same design was also used in all subsequent studies on pyraclostrobin, unless otherwise indicated.

For assessments of radial growth, dishes were inoculated with a 5-mm agar plug cut from the margin of an actively growing culture using a cork borer. The inoculated dishes were incubated at 12/12 h light/dark period and 20° ± 2° C for 2 weeks (Wise *et al.*, 2009). The width of each colony at its widest point and perpendicular to the first measurement was measured at 7 and 14 days after inoculation. The pattern of response was more consistent at 14 days, so only that data is presented. The mean colony radius was determined by subtracting the diameter of the initial 5-mm plug, and dividing by two. The percentage of growth inhibition relative to the control was calculated as: [1 – (growth on amended medium / growth of control)] x 100.

For assessments of conidial germination, conidial suspensions were prepared from 7- to 10-day-old cultures of each isolate grown on half-strength oatmeal agar. The concentration was assessed using a haemocytometer and adjusted to 1 x 10^5 conidia mL^{-1}. Aliquots of conidial suspension (100 µL) of each isolate were pipette onto Petri dishes containing pyraclostrobin, with or without SHAM, and the drops containing conidia were dispersed across the surface with gentle shaking. After 24 h of incubation in the dark at 20° ± 2° C, 100 conidia per plate were assessed microscopically (100 x magnification) and evaluated for germination. A conidium was considered germinated if the germ tube was at least equal in length to the conidium or if there were multiple germ tubes developing normally from a single conidium. The percentage of germination inhibition

relative to the control was calculated, as: [1 – (germination on amended medium / germination of control)] x 100.

Single-spore and mass-transfer isolates: Three sensitive, four intermediate, three insensitive isolates of *A. rabiei* from 2007 were selected for this trial, as described previously. Four single-spore isolates were developed from each mass-transfer isolate. The single-spore isolates were obtained by spreading a drop of dilute conidial suspension across the surface of a Petri dish filled with water agar, then identifying single germinated conidia under a microscope and transferring the individual conidia to fresh dishes of agar media. Dishes of PDA were amended with formulated pyraclostrobin (Headline), SHAM (dissolved in methanol at 0.1% by volume) was added at 100 $\mu g\ mL^{-1}$ and radial growth was assessed as described previously. Control treatments were amended with SHAM. A discriminatory dose of 5 μg pyraclostrobin mL^{-1} was chosen for the study based on the results of previous experiments.

Formulated product and technical grade pyraclostrobin: The trial was conducted using PDA amended with formulated pyraclostrobin (Headline) or technical grade pyraclostrobin at 5 $\mu g\ mL^{-1}$ with the addition of SHAM (100 $\mu g\ mL^{-1}$). Radial growth of the same 10 isolates that represented categories of sensitivity to *A. rabiei* from 2007 was assessed as described previously. Headline and pyraclostrobin were dissolved in acetone. The control treatment was amended with acetone and SHAM.

Data analysis: All of the statistical analyses were conducted using SAS, and analysis of variance was conducted using PROC MIXED. Differences are significant at $P = 0.05$ unless otherwise stated. The repetitions of each experiment did not differ, so the data were pooled for subsequent analyses. The inhibition of radial growth or conidial germination of each isolate was determined as described previously. The reduction of radial growth or conidial germination (%) for each isolate in each replicate in relation to the $\log_{10}$ of the fungicide concentration was used in PROC PROBIT to calculate the EC_{50} values (the concentration required to inhibit 50% of radial growth or conidial germination).

In the study to assess the effect of SHAM in assessments of pyraclostrobin sensitivity, SHAM and isolate category (sensitive, intermediate, insensitive) were fixed factors, and repetition, isolate nested within category and isolate x SHAM interaction were random factors. The response variable was the EC_{50} value calculated for each replicate. The EC_{50} values were then subjected to analysis of variance followed by separation of least squares means using Tukey's multiple means comparison test at $P= 0.05$. A correlation analysis of response based on the EC_{50} values of the two assessment techniques with and without SHAM (combined across repetitions) was conducted using PROC CORR to determine if the same pattern of response was observed for both assessment techniques.

The nature of the response of individual isolates from single-spore and mass-transfer isolates to the discriminatory dose of pyraclostrobin was determined using single-degree-of-freedom contrasts within a mixed model analysis of variance. Growth inhibition of the four single-spore colonies of each isolate was compared to that of the original mass-transfer isolate. Isolate category was a fixed factor, and repetition and isolate within isolate category were random factors in this analysis.

The impact of fungicide formulation on isolate sensitivity was assessed using a mixed model analysis of variance. Isolate category was a fixed factor, and repetition and isolate within isolate category were random factors in this analysis.

RESULTS

Effect of SHAM on radial growth and conidial germination: Data were analyzed from both assessment dates (7 and 14 days), but colony growth at 14 days was used for analysis of the effects of pyraclostrobin on radial growth to allow sufficient time to develop significant differences between amended and nonamended media. It was possible to observe differences in sensitivity to pyraclostrobin among the 10 isolates of *A. rabiei* in the trials that assessed radial growth and conidial germination with and without the addition of SHAM. The individual EC_{50} values for radial growth of isolates cultured without SHAM ranged from 0.5 to 15 μg mL^{-1}, with a mean of 5.8 μg mL^{-1}, and from 0.3 to 11 μg mL^{-1}, with a mean of 4.0 μg mL^{-1} with SHAM. The individual EC_{50} values for conidial germination of isolates cultured without SHAM ranged from 0.001 to 0.4 μg mL^{-1}, with a mean of 0.19 μg mL^{-1}, and from 0.001 to 0.2 μg mL^{-1}, with a mean of 0.09 μg mL^{-1} with SHAM (Table 1). SHAM reduced EC_{50} values for both radial growth and conidial germination, but the effect was small, there was isolate x SHAM interaction in both assessments, but addition of SHAM did not affect the differentiation of sensitive versus insensitive isolates (Figs. 5, 6).

Sensitive isolates were readily separated from intermediate and insensitive isolates, but there was no consistent difference between intermediate and insensitive isolates in either assay (Table 2, Figs. 5, 6). Within each testing technique (radial growth and conidial germination), there was a strong and highly significant positive correlation of isolates assessed with and without SHAM. The correlations were not as strong (but still highly significant) across techniques (Table 2, Figs. 7, 8). A discriminatory dose of 5 μg mL^{-1} of the Headline formulation, without the addition of SHAM for radial growth assessments, was selected for subsequent assessments of strobilurin sensitivity. This discriminatory dose was selected above the mean EC_{50} value found in the inhibition of growth of *A. rabiei* by pyraclostrobin where 90% of the isolates fall within the EC_{50} range and 10% above to allow for the detection of highly insensitive isolates. Also, a discriminatory dose of 5 μg a.i mL^{-1} clearly differentiated the sensitive and insensitive isolates in these assessments based on radial growth assay.

Table 1. Effect of salicylhydroxamic acid (SHAM) on sensitivity to pyraclostrobin (mean EC_{50} µg mL^{-1}) of selected isolates of *Ascochyta rabiei* in radial growth and conidial germination assays.

Isolate category	Radial growth EC_{50} (µg mL^{-1})		Conidial germination EC_{50} (µg mL^{-1})	
	+ SHAM	- SHAM	+ SHAM	- SHAM
Insensitive				
Jul08132	10.9 bc	15.2 a	0.2 d	0.4 a
Jul08125	9.6 c	12 b	0.1 de	0.3 bc
Jul0866	4.2 e	7.0 d	0.1 de	0.3 ab
Intermediate				
Jul0864	4.5 e	7.2 d	0.1 e	0.3 bc
Jul0880	3.1 e	4.1 e	0.1 de	0.3 c
Jul08120	2.9 ef	4.4 e	0.1 de	0.3 a
Jul0871	2.6 efg	3.9 e	0.1 de	0.3 c
Sensitive				
Jul08124	0.3 h	0.6 fgh	0.002 f	0.003 f
Jul0878	0.3 h	0.6 gh	0.002 f	0.002 f
Jul0861	0.3 gh	0.5 gh	0.001 f	0.001 f

+ = with SHAM, - = without SHAM.
(*a–f*) Values within a column followed by the same letter do not differ based on Tukey's multiple means comparison test at $P = 0.05$.

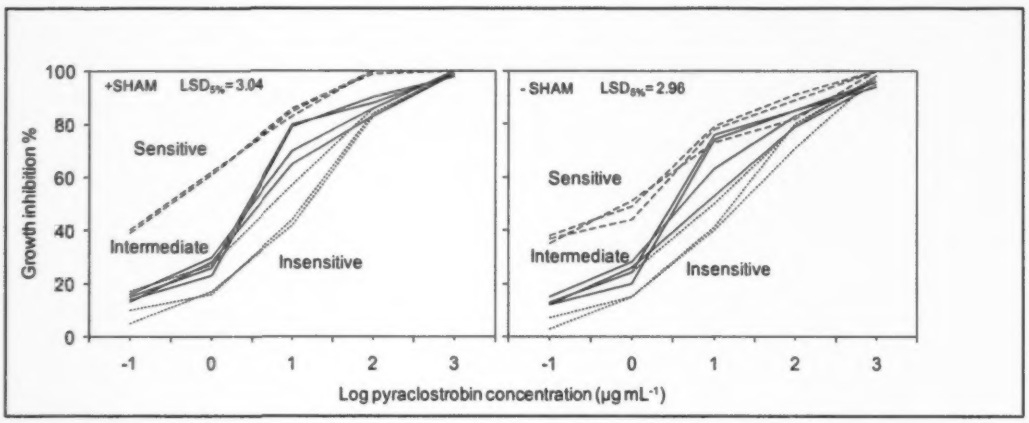


Fig. 5. Inhibition of radial growth by pyraclostrobin with (+) and without (-) SHAM in sensitive, intermediate, and insensitive isolates of *Ascochyta rabiei* (n = 6). Each line represents one isolate.

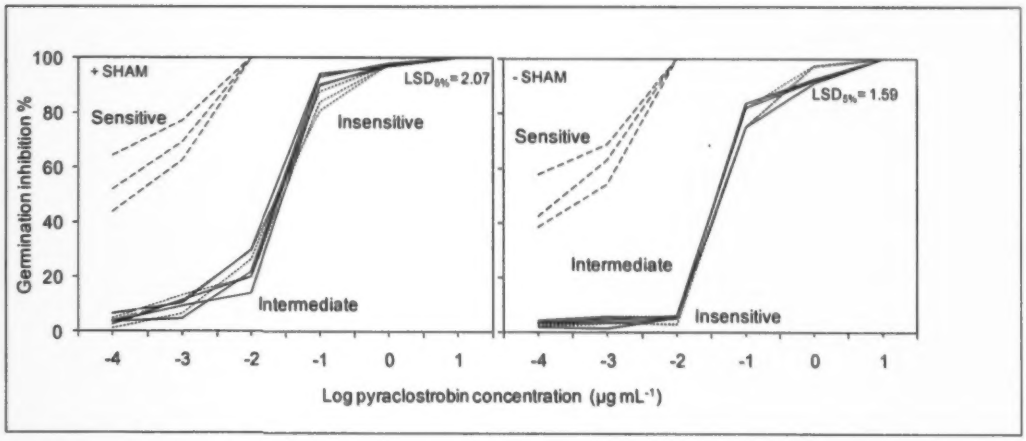


Fig. 6. Inhibition of conidial germination by pyraclostrobin with (+) and without (-) SHAM in sensitive, intermediate, and insensitive isolates of *Ascochyta rabiei* (n = 6). Each line represents one isolate.

Table 2. Coefficient of correlation (r) for assessment of QoI sensitivity in *Ascochyta rabiei* based on radial growth (RG) and conidial germination (CG) assessments with (+) and without (-) SHAM (n = 12).

Assessment technique	RG - SHAM	CG + SHAM	CG - SHAM
RG + SHAM	0.97[a] <0.0001[b]	0.67 <0.0001	0.61 0.0004
RG – SHAM		0.70 <0.0001	0.63 0.0002
CG + SHAM			0.88 <0.0001

[a] r = correlation coefficient.
[b] *P* value for the correlation coefficient.

Single-spore versus mass-transfer isolates: The response pattern (sensitive, intermediate, or insensitive) of isolates assessed as the original mass-transfer culture or derived single-spore cultures did not differ substantially for any isolate. However, there were small differences in response for individual isolates. There were no differences in the response of single-spore and mass-transfer cultures among the insensitive isolates, but there was a difference for one of the three sensitive isolates and for three of the four intermediate isolates. For the sensitive isolate where a difference was observed, the single-spore isolates were more sensitive to pyraclostrobin than the mass-transfer isolate. The single-spore isolates were less sensitive than the mass-transfer isolates from which they were derived in three of the four intermediate isolates (Fig. 9).

Technical grade pyraclostrobin versus formulated product: There were no differences in inhibition of radial growth by the formulated product (Headline EC) o technical grade pyraclostrobin at the discriminatory dose of 5 µg a.i. mL^{-1} (Fig. 10).

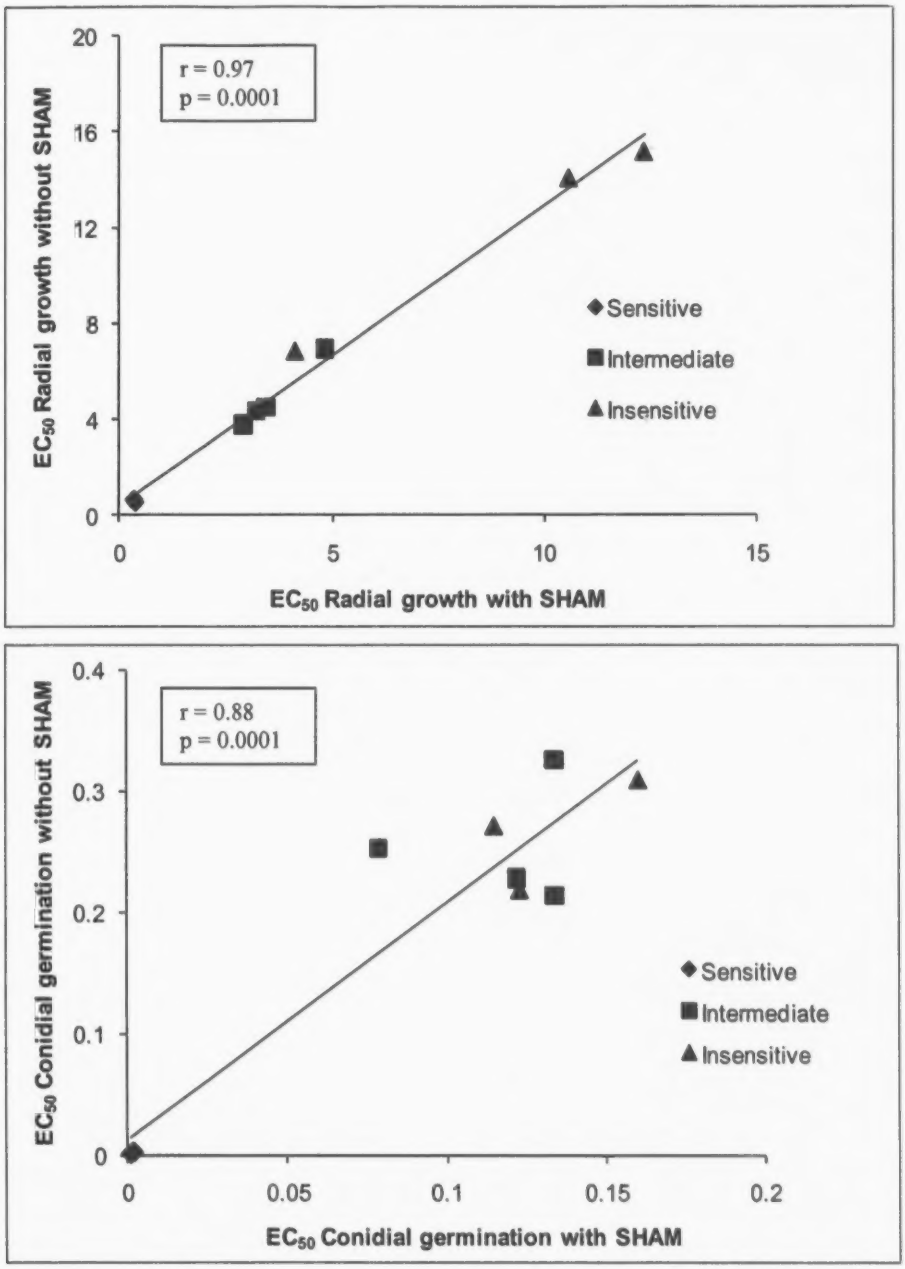


Fig. 7. Correlation of EC_{50} values for *Ascochyta rabiei* isolates assessed with and without SHAM within each assessment technique. Each point is the mean for one isolate.

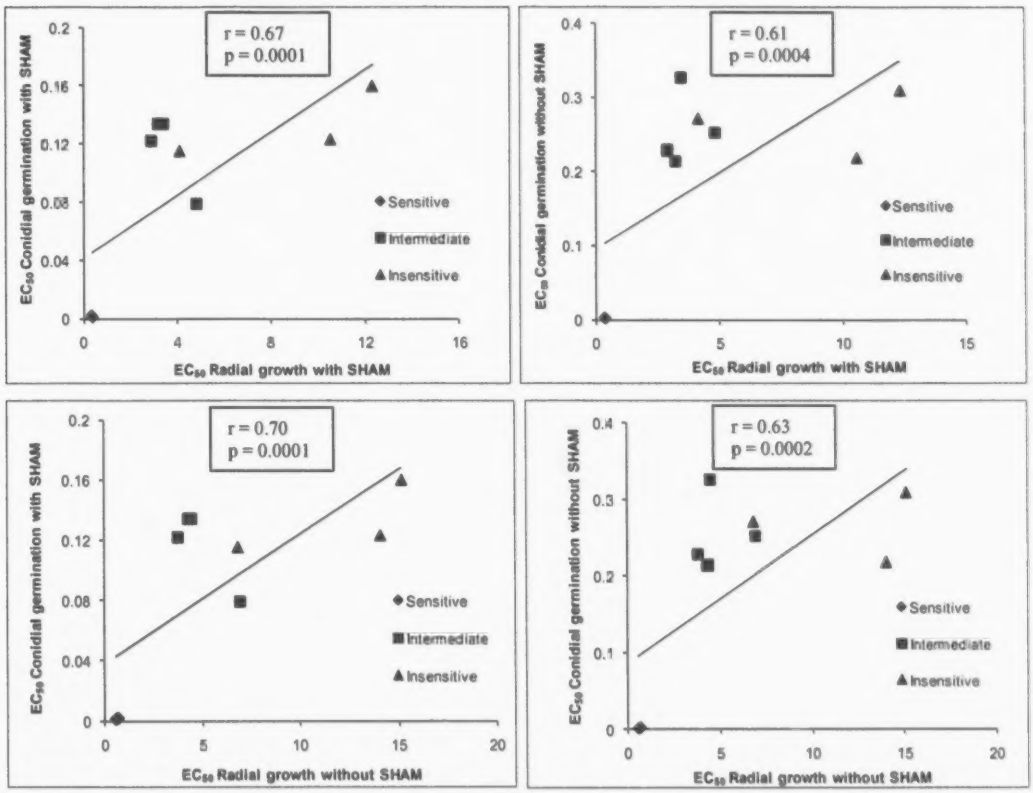


Fig. 8. Correlation of EC_{50} values for *Ascochyta rabiei* isolates assessed with and without SHAM across assessment techniques (radial growth vs. conidial germination). Each point is the mean for one isolate.

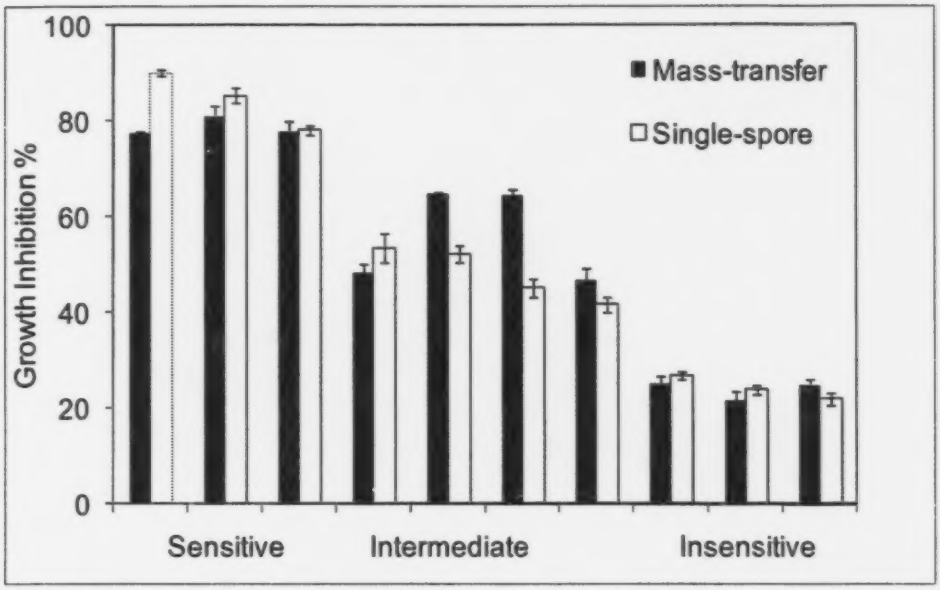


Fig. 9. Growth inhibition of pyraclostrobin (5 μg mL^{-1}) in single-spore derived vs. mass-transfer isolates of sensitive, intermediate, and insensitive isolates of *Ascochyta rabiei*, compared to unamended PDA. Error bars represent standard error (n = 6) at $P = 0.05$.

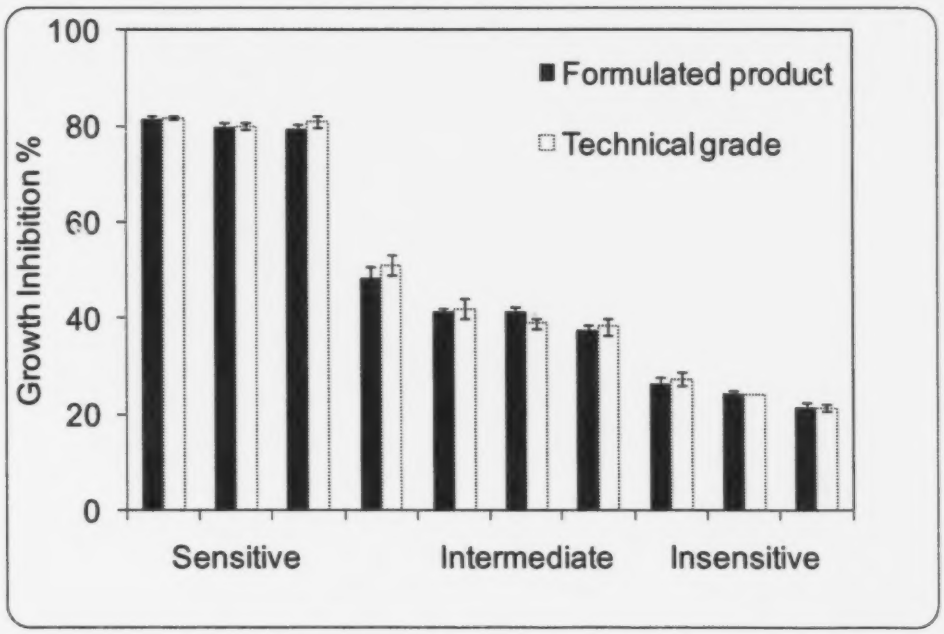


Fig. 10. Effects of technical grade active ingredient and formulated fungicide on sensitivity to pyraclostrobin in sensitive, intermediate, and insensitive isolates of *Ascochyta rabiei,* based on inhibition in radial growth at 5 μg mL^{-1}. Error bars represent standard error (n = 6) at $P = 0.05$.

Discussion

This portion of the current study was conducted to develop a simple and effective method of determining the sensitivity to pyraclostrobin in isolates of *A. rabiei*. In addition, these studies provide information on factors of interest for the development of routine assessment protocols for other pathogens and for use in diagnostic laboratories.

Some fungal species are able to use an alternative respiratory pathway to bypass the effect of QoIs at the quinol-oxidizing site. This allows them to germinate and grow *in vitro* in the presence of high levels of QoI fungicides, even though the process of pathogen infection and growth based solely on this pathway is generally too slow to cause disease under field conditions. SHAM can be used to block this alternative respiratory pathway and permit a more accurate *in vitro* assessment of the ability of the pathogen population to cause disease in the presence of the QoI fungicide.

In a detailed comparison of 10 isolates selected to represent a range of sensitivity response, sensitive isolates were readily separated from intermediate and insensitive isolates in both assay systems. In the radial growth assay, the intermediate isolates showed a similar pattern of response to that of the insensitive isolates, but were not as tightly aligned as in the conidial germination assessments. However, there was a clear separation between the intermediate and sensitive isolates within the range of 1–10 μg mL^{-1}. We conclude that the isolates that had initially been identified as having intermediate sensitivity should be characterized as insensitive to QoI fungicides for routine assessment

SHAM reduced radial growth and conidial germination slightly (with or without a strobilurin fungicide), but did not improve the differentiation of sensitive and insensitive isolates. This result supports a previous report (Wise et al. 2008) that some isolates of *A. rabiei* can use the alternative respiration pathway. The EC_{50} values for radial growth were much higher than for conidial germination assessments, but both techniques showed the same pattern of response. There was an isolate x SHAM interaction for both techniques, but these small interactions did not affect the differentiation of sensitive versus insensitive isolates because the effect of SHAM was small compared to the large difference between sensitive and insensitive isolates. SHAM is not generally available in testing labs, and so should only be included in routine testing protocols if it adds value to the test. We do not believe that this is the case, and so SHAM is not recommended for routine sensitivity assessment of *A. rabiei* to pyraclostrobin.

Previous studies had utilized both radial growth and conidial germination assays for assessment of sensitivity to QoIs in *A. rabiei* (Chang *et al.*, 2007; Gossen and Anderson, 2004; Wise et al. 2008, 2009). In the current study, EC_{50} values for pyraclostrobin in the radial growth assessments were substantially higher than in the conidial germination assay, but both techniques showed the same pattern of response. Within a testing technique, there was a strong and highly significant positive correlation in the response of isolates assessed with and without SHAM. The correlations were not as strong, but still highly significant, across techniques. We conclude that radial growth assessments were as effective as assessments of conidial germination for quantifying insensitivity to pyraclostrobin, but that assessments based on radial growth are easier and less time-

consuming to conduct than conidial germination assays, although more time is required to obtain the results.

Technical grade active ingredient of commercial fungicides is not easy to obtain because it is only available from the commercial manufacturer, who demand tight controls of quantities and usage. As a result, it is not readily available to diagnostic laboratories and should only be included in a testing protocol if it is essential for the assessment. There were no differences in inhibition of radial growth of any of the isolates grown on agar amended with the formulated product (Headline) as compared to the technical grade of pyraclostrobin, so we conclude that use of technical grade active ingredient is not essential for accurate testing of sensitivity to QoIs in *A. rabiei*. Therefore, use of formulated product is recommended for routine assessments.

Single-spore isolates are generally more homogeneous than mass-transfer isolates, which may contain a mixture of two or more genotypes of the pathogen. The pattern of response in strobilurin sensitivity in single-spore versus mass-transfer isolates did not differ for highly sensitive and highly insensitive isolates. However, single-spore isolates from mass-transfer isolates in the intermediate class exhibited a larger range of response than the other groups, which may indicates that the mass-transfer isolates were more genetically heterogeneous than the single-spore isolates. We conclude that single-spore isolates would provide a more accurate assessment, but that using mass-transfer isolates would result in substantial saving in time, labour, and expense with only a small sacrifice in accuracy for routine assessments in areas where insensitivity is know to occur at moderate to high levels. Therefore, assessment of mass transfer isolates is recommended.

Monitoring for changes in fungicide sensitivity based on a single discriminatory dose allows for rapid assessment of a large number of isolates and has been used for several plant pathogens. A discriminatory dose of 5 μg a.i mL^{-1} as Headline to assess the sensitivity of isolates collected in 2008 in Saskatchewan was selected because it clearly differentiates the sensitive and insensitive isolates, as demonstrated in the study on the effect of SHAM and pyraclostrobin on radial growth. This concentration provided a satisfactory separation of sensitive and insensitive isolates in the current study, and we recommend that a discriminatory dose of between 1 and 5 μg a.i mL^{-1} be used in a future monitoring program for detection of insensitivity of *A. rabiei* to pyraclostrobin using a radial growth assay.

The methods examined in this portion of the study can be combined into a protocol to provide a simple and effective screening test to assess sensitivity to strobilurin fungicides in *A. rabiei* that can be used by diagnostic laboratories and other general laboratory settings. We recommend the use of a radial growth assay without SHAM, mass-transfer isolates (for routine assessments), and formulated product (Headline) because this combination would provide substantial savings in time, labour, and expense. This method will likely not be used extensively in western Canada because insensitivity in *A. rabiei* is already so widespread that little further testing for QoI sensitivity is anticipated. However, it could be used in other regions where insensitivity has not developed as extensively. For rapid assessment of population sensitivity (e.g., where a loss of sensitivity is being suspected in a crop during the growing season), assessment of conidial germination using conidia collected directly from fresh infected leaves could be considered.

C. EVALUATION OF EFFICACY OF REGISTERED ACTIVES

MATERIALS: Bravo 500 (chlorothalonil, Syngenta Crop Protection Canada, Inc., Guelph, ON); Quadris (azoxystrobin, 250g/L; Syngenta Crop Protection Canada Inc.) Headline EC (pyraclostrobin, BASF Canada Inc., Toronto, ON); Lance 70% DF (boscalid, BASF Canada Inc.), Proline 480 SC (prothioconazole, Bayer CropScience Inc., Calgary, AB)

METHODS: A trial to assess the efficacy of several new fungicides to control ascochyta blight of chickpea was conducted at the AAFC Research Farm at Saskatoon in 2007 on a heavy clay-loam soil. The kabuli chickpea CDC Frontier was selected for the trial. It has fair ascochyta blight resistance (the best available), a fern leaf type and late maturity (Saskatchewan Agriculture and Food, 2007). The trial was seeded on 15 May. The plots were direct seeded at about 45 plants per m^2 using a double-disc drill seeder. The layout was a randomized complete block design (RCBD) with four replications. Each plot consisted of eight rows, each 6-m long, with 0.3 m between rows and 1.2 m between plots, separated by wheat guard rows. The weed control consisted of applying Edge herbicide (ethalfluralin, Dow AgroSciences Canada Inc., Calgary, AB) together with11-51-0 fertilizer in early spring before seeding, hand tillage of plots at seedling stage, and then hand weeding as needed.

There were 10 treatments: 1) Bravo at 1.5 kg a.i./ha/ Quadris at 124 g a.i./ha/ Bravo, 2) Bravo/ Lance at 294 g a.i./ha, Bravo, 3) Bravo/ Proline at 200 g a.i./ha/ Bravo, 4) Bravo/ Headline at 100 g a.i./ha/ Lance, 5) Headline/ Bravo/ Bravo, 6) Bravo/ Bravo/ Headline, 7) Proline/ Headline/ Bravo, 8) Bravo/ Proline/ Headline, 9) Bravo/ Headline/ No spray (Control 1), and 10) Bravo/ Headline/ Bravo (Control 2).The fungicides were applied with a CO_2 backpack sprayer equipped with four XR TeeJet 8002 VS nozzles spaced 45 cm apart and calibrated to deliver 250 L/ha at 240 kPa.

In 2008, the initial fungicide treatments were applied on 23 July at early flower to flowering stage. A second application was made at late flowering to early pod stage on 03 August, and a third application at pod stage on 16 August. Ten plants per plot were selected at random and assessed for ascochyta blight severity on whole plants (foliage and stems) using the Horsfall-Barratt scale (0–11) on 24 July, 08 August and 04 September. The trial was desiccated on 14 September and was harvested on 02−03 October using a small-plot combine. Seed was air-dried, cleaned, and weighed, and yield was determined.

The trial was repeated in 2008; seeded 16 May, fungicides applied 05, 18, and 28 August, ascochyta blight rated on 01, 20, 29 August and 11 September, and harvested 23−24 October. Headline was replaced with Headline Duo (a combination of pyraclostrobin and boscalid) because the pathogen population was expected to be (or quickly become) insensitive to the strobilurin alone.

In 2009, the study was repeated, but extreme drought at seedling resulted in delayed germination and extremely uneven stands in this trial. As a result, the test was abandoned. In 2010, record rainfall in spring resulted in flooding that persisted for

several weeks in many areas. The plant stand was lost to drowning and root disease, and the trial was abandoned.

RESULTS

Prior to treatment application in both 2007 and 2008, blight severity was low and there were no differences among treatments (Tables 3, 4). In 2007, severity had increased slightly by 08 August, but there were no differences among treatments. On 04 September, severity had increased slightly, resulting in small differences among treatments. The final yields showed significant differences, but there was no clear pattern with regard to fungicide use due to low disease pressure (Table 3).

In 2008, severity had increased substantially by 20 August, and there were differences among treatments. At subsequent rating dates, severity increased only slightly and differences among the treatments were small. Fungicide application treatments did not affect seed yield, probably as a result of the low levels of blight severity (Table 4).

Table 3. Impact of fungicide application on severity (0–11 scale) of ascochyta blight and seed yield of chickpea at Saskatoon in 2007.

Fungicide combinations	Severity			Yield (Mg/ha)
	Jul. 24	Aug. 08	Sept. 04	
Bravo / Quadris / Bravo	0.95 a[1]	1.7 a	2.0 abc	1.57 ab
Bravo / Lance / Bravo	0.95 a	1.5 a	1.9 abc	1.74 ab
Bravo / Proline / Bravo	0.82 a	1.7 a	2.2 ab	1.83 ab
Bravo / Headline / Lance	0.72 a	1.7 a	2.1 ab	1.87 ab
Headline / Bravo / Bravo	0.87 a	1.4 a	2.1 abc	0.81 b
Bravo / Bravo / Headline	0.67 a	1.4 a	2.0 abc	2.22 a
Proline / Headline / Bravo	0.65 a	1.4 a	1.8 bc	1.38 ab
Bravo / Proline / Headline	0.87 a	1.2 a	2.0 abc	1.38 ab
Bravo / Headline / no spray (Control 1)	0.67 a	1.5 a	2.4 a	1.17 ab
Bravo / Headline / Bravo (Control 2)	0.67 a	1.5 a	1.5 c	1.08 ab

[1] Means in a column followed by the same letter do not differ at $P = 0.05$ based on the Waller-Duncan K-ratio t test.

Table 4. Impact of fungicide application on severity (0–11 scale) of ascochyta blight and seed yield of chickpea at Saskatoon in 2008.

Fungicide combinations	Severity				Yield
	Aug. 01	Aug. 20	Aug. 29	Sept. 11	(Mg/ha)
Bravo / Quadris / Bravo	1.9 a[1]	3.6 de	2.9 ab	3.1 b	1.68 a
Bravo / Lance / Bravo	1.9 a	4.2 a	2.8 ab	3.1 b	1.68 a
Bravo / Proline / Bravo	2.1 a	4.2 a	3.1 ab	3.2 b	1.92 a
Bravo / Headline / Lance	2.0 a	3.8 c	2.9 ab	3.2 b	1.52 a
Headline / Bravo / Bravo	2.0 a	3.7 cd	2.8 ab	3.3 b	1.84 a
Bravo / Bravo / Headline	2.1 a	4.1 ab	2.9 ab	3.0 b	1.66 a
Proline / Headline / Bravo	1.9 a	3.5 e	2.7 b	3.2 b	1.90 a
Bravo / Proline / Headline	1.9 a	3.9 b	2.9 ab	3.0 b	1.76 a
Bravo / Headline / no spray (Control 1)	2.0 a	3.7 cd	3.2 a	3.7 a	1.48 a
Bravo / Headline / Bravo (Control 2)	2.0 a	3.9 b	2.8 ab	3.8 a	1.75 a

[1] Means in a column followed by the same letter do not differ at $P = 0.05$ based on the Waller-Duncan K-ratio t test.

CONCLUSIONS: When the fungicide treatments were initiated in both 2007 and 2008, levels of ascochyta blight were low, but the disease was present in each plot. This indicates that inoculum was present in the area. However, conditions were not conducive for blight development, and the epidemic never developed to severe levels in either year.

In both years, differences in blight severity associated with application of the various fungicide combinations were small and inconsistent. In 2007, treatments had only a small impact on severity and seed yield. In 2008, application of fungicides consistently reduced blight severity, but did not affect seed yield. For both years, the small impact of fungicide application on severity of ascochyta blight that occurred as a result of weather conditions that were not conducive to blight increase resulted in small and inconsistent impacts on yield.

Collaborators have conducted a repetition of this trial in Alberta, which was summarized in a report to the Alberta Pulse Growers. Dr. Banniza and colleagues at the U of S have a separate grant to address the issue of fungicide efficacy in rotation directly and in more detail than was possible as a small component of the current study, and so no further work is planned in this area.

C. FIELD EVALUATION OF NOVEL ACTIVES

C.1 Penthiopyrad

Field trials to assess the efficacy of penthiopryad, a new and as yet unregistered succinate dehydrogenase inhibitor (SDHI) fungicide active from DuPont were conducted from 2007 to 2010. A registration package for this active has been submitted by the company

Field trials 2007–2008

MATERIALS AND METHODS

Penthiopryad EC and SC (E.I. DuPont Canada Co., Mississauga, ON); Bravo 500 (chlorothalonil, Syngenta Crop Protection Canada, Inc., Guelph, ON); Quadris (azoxystrobin, 250g/L; Syngenta Crop Protection Canada Inc.); Manzate 200 DF (mancozeb, E.I. DuPont Canada Co.) ; Lance 70% DF (boscalid, BASF Canada Inc., Toronto, ON).

Field trials to assess the impact of foliar fungicide application on ascochyta blight severity and seed yield of chickpea cv. CDC Xena were conducted on a Sutherland clay-loam soil at the AAFC Research Farm at Saskatoon in 2007 and 2008. CDC Xena has a unifoliate leaf type, late maturity, and is highly susceptible to ascochyta blight (Saskatchewan Agriculture and Food, 2007). The seed was treated with Allegiance FL (metalaxyl, Bayer CropScience Inc. Calgary, AB) at 0.016 L/100 kg of seed before seeding to control seedling blight caused by *Pythium* spp. The plots were direct seeded at about 45 plants per m^2 using a double-disc drill seeder. The trial was seeded on 11 May in 2007. In 2008, a portion of the trial was seeded on 16 May and the remainder on 23 May.

The studies were laid out as a RCBD with four replications. Plot layout and management were as described previously. There were 14 treatments in the trial in 2007, with each applied at the pre-bloom and mid-bloom stage: penthiopryad EC at 100, 150, 200, 250 and 300 g a.i./ha, penthiopryad SC at 100, 150, 200, 250 and 300 g a.i./ha, Manzate 200 DF at 1.7 kg a.i./ha, Lance at 290 g a.i./ha; and Bravo 500 at 1.5 kg a.i./ha at pre-bloom stage and Quadris at 120 g a.i./ha at mid-bloom stage, plus an untreated control. In 2008, treatments penthiopryad EC and SC at 150 g a.i./ha and Manzate 200 DF at 1.7 kg a.i./ha were dropped from the trial. The treatments were applied with a CO_2 backpack sprayer (Model GS, R&D Sprayers, Inc.) equipped with an aluminum spray boom with four XR TeeJet 8002 VS nozzles spaced 48 cm apart, calibrated to deliver 250 L/ha at 240 kPa. The fungicide treatments were applied at early flowering (13 July, 2007 and 29 July, 2008) and early pod stage (27 July, 2007 and 14 August, 2008).

Ten plants per plot were selected at random and assessed for ascochyta blight severity on whole plants (foliage and stems) using the Horsfall-Barratt scale (0–11) on 12 and 26 July, and 13 August in 2007 and 28 July and 12 and 29 August in 2008. Crop tolerance (% foliar damage/injury) was assessed on July 26 and August 13 in 2007, and 12 and 29 August in 2008; no injury was observed on any plot. The trial was harvested on 10–11 September in 2007 and 22–23 October in 2008. The seed was air-dried and cleaned, then weighed. Analysis of variance (General Linear Model Procedure of SAS) was conducted

to assess the impact of treatments, with means separation using the Waller-Duncan K-ratio t test. Differences are significant at $P = 0.05$ unless specifically noted.

RESULTS

Seedlings emerged quickly and uniformly in 2007 and plant growth stage was uniform across the trial. In 2008, soil conditions were so dry that most of the seedlings did not emerge until early June, after rains in late May. This resulted in uneven stands throughout the season and increased variability in assessments of both foliar disease and yield.

When the fungicide treatments were initiated, levels of ascochyta blight were low, but the disease was present in all plots in both years. This indicates that natural inoculum for ascochyta was present at the site. At the first rating date (prior to treatment initiation), severity was low and there were no differences among treatments in either year (Tables 5, 6). This low level of ascochyta blight indicates that conditions early in the growing season were not conducive for disease development in either year. At the 2nd rating date, severity had increased substantially. There were differences in severity between the treatments and the control in 2007 (Table 5), but no difference in 2008 (Table 6). At the 3rd rating date, there were differences among treatments in both 2007 and 2008, with the control having the highest severity. These differences were reflected in seed yield, with the fungicide treatments having higher yields than the control. Yield was quite variable in 2008, likely because of uneven seedling establishment during dry conditions in May. Also, yields were about twice as high in 2007 as 2008.

Table 5. Impact of two applications of foliar fungicide on severity of ascochyta blight (0–11 scale) and seed yield of chickpea cv. Xena at Saskatoon in 2007.

Fungicide treatment	Rate (g a.i./ha)	Blight severity (0–11 scale)			Yield
		Jul. 12	Jul. 26	Aug. 13	(Mg/ha)
Nontreated control	---	2.0 a	3.3 a	4.7 a	1.97 b
Penthiopryad EC	100	1.9 a	2.7 b	3.9 b	2.40 ab
	150	1.7 a	2.6 bc	3.7 bc	2.61 a
	200	1.7 a	2.4 cde	3.1 f	2.52 a
	250	1.6 a	2.6 bc	3.3 c-f	2.59 a
	300	1.7 a	2.5 bcd	3.2 ef	2.58 a
Penthiopryad SC	100	1.7 a	2.3 de	3.4 c-f	2.11 ab
	150	1.8 a	2.5 bcd	3.2 def	2.50 a
	200	1.8 a	2.4 cde	3.6 cd	2.61 a
	250	1.8 a	2.4 cde	3.1 f	2.39 ab
	300	1.7 a	2.3 de	3.2 def	2.51 a
Bravo + Quadris	1500/124	1.6 a	2.2 de	3.1 f	2.59 a
Manzate	1700	1.8 a	2.4 b-d	3.4 cde	2.15 ab
Lance	300	1.7 a	2.1 e	3.1 f	2.60 a

(*a–f*) Means in a column followed by the same letter do not differ at $P = 0.05$ based on the Waller-Duncan K-ratio t test.

Table 6. Impact of two applications of foliar fungicide on severity of ascochyta blight (0–11 scale) and seed yield of chickpea cv. Xena at Saskatoon in 2008.

Fungicide treatment	Rate (g a.i./ha)	Blight severity (0–11 scale)			Yield
		Jul. 28	Aug. 12	Aug. 29	(Mg/ha)
Nontreated control	---	2.6 a	5.8 a	6.7 a	0.82 c
Penthiopryad EC	100	2.5 a	5.4 a	5.4 b	1.02 bc
	200	2.2 a	5.3 a	5.2 bc	1.30 abc
	250	2.3 a	5.1 a	4.9 d	1.37 abc
	300	2.7 a	5.4 a	5.0 cd	1.59 ab
Penthiopryad SC	100	2.6 a	5.6 a	5.3 b	1.02 bc
	200	2.6 a	5.2 a	5.2 bc	1.78 a
	250	2.4 a	5.4 a	5.0 cd	1.32 abc
	300	2.5 a	5.3 a	5.0 cd	1.27 abc
Bravo + Quadris	1500/120	2.5 a	5.6 a	5.3 b	1.66 a
Lance	290	2.4 a	5.4 a	4.9 cd	1.36 abc

(*a–f*) Means in a column followed by the same letter do not differ at $P = 0.05$ based on the Waller-Duncan K-ratio t test.

Field trials 2009–2010

MATERIALS AND METHODS

2009: penthiopryad EC, DPX-YT669, DPX-Q8X63-001, and DPX-Q8X63-002 (E.I. DuPont Canada Co., Mississauga, ON); Bravo 500 (chlorothalonil, Syngenta Crop Protection Canada, Inc., Guelph, ON); Quadris (azoxystrobin, 250g/L; Syngenta Crop Protection Canada Inc.); Lance 70% DF (boscalid, BASF Canada Inc., Toronto, ON).

A trial to assess the impact of foliar fungicide application on ascochyta blight severity and seed yield of chickpea cv. CDC Frontier was conducted on a Sutherland clay-loam soil at the AAFC Research Farm at Saskatoon in 2009. CDC Frontier is a kabuli chickpea with a fern leaf type, late maturity and has fair ascochyta blight resistance (Saskatchewan Agriculture and Food, 2009). The plot area had been seeded to barley the previous year.

The studies were laid out in a RCBD with four replications. Plot layout and management were as previously described. The plots were direct seeded on 26 May, 2009 at about 45 plants per m^2 using a double-disc drill seeder. Germination was delayed and irrigation was required as a result of drought conditions in spring. Overhead irrigation was applied on 12, 15 and 16 June (2.4, 1.5, and 1.0 cm of water applied, respectively) using a riser and impact nozzle system. Chickpea residue that had been collected in 2008

from a crop that was heavily infested with ascochyta blight was applied evenly to the plot area on 28 July to initiate an epidemic of ascochyta blight.

There were nine fungicide treatments in the trial, each applied at early bloom (August 10) and late bloom (August 25) stage: penthiopryad EC applied at 150 and 250 g a.i./ha; DPX-YT669 at 150 g a.i./ha; DPX-Q8X63-002 at 300 g a.i./ha; DPX-Q8X63-001 at 200, 300 and 400 g a.i./ha; Lance at 290 g a.i./ha; Bravo 500 at 1.5 kg a.i./ha at early bloom followed by Quadris at 120 g a.i./ha at late bloom, and a nontreated control. The fungicides were applied at 240 kPa in 250 L/ha spray volume using a CO_2 backpack sprayer (Model GS, R&D Sprayers, Inc.) equipped with an aluminum spray boom and four XR TeeJet 8002 VS nozzles spaced 48 cm apart.

Ten plants per plot were selected at random and assessed for ascochyta blight severity on whole plants (foliage and stems) on 11, 22 August and 04 September. Crop tolerance was assessed on 22 August and 04 September; no injury was observed in any plot. The trial was not harvested, because the plants were killed by frost before the seed matured. Analysis of variance (General Linear Model Procedure of SAS) was conducted on the disease ratings and means were separated using the Waller-Duncan K-ratio T test at $P = 0.05$.

2010: Penthiopyrad EC and picoxystrobin (E.I. DuPont Canada Co., Mississauga, ON); Lance 70% DF (boscalid, BASF Canada Inc., Toronto, ON).

A trial to assess the impact of foliar fungicide application on ascochyta blight severity and seed yield of chickpea cvs. CDC Frontier and CDC Xena was conducted on a Sutherland clay-loam soil at the AAFC Research Farm at Saskatoon in 2010. Seeding was delayed by heavy rain and cool conditions. Several early plantings were drowned out, and even the last seeding date did not establish well. As a result, the best plots from each of two adjacent trials were combined into one test with two reps of CDC Frontier and two reps of CDC Xena.

The plots were direct seeded at about 45 plants per m^2 using a double-disc drill seeder on 15–16June. Plot layout and management were as previously described Crop residue collected in 2009 from a chickpea field that was heavily infested with ascochyta blight was applied evenly to the plot area on July 28 to initiate the blight epidemic.

Seven foliar fungicide treatments were applied on August 19 at the mid-bloom stage: penthiopyrad EC @ 200 g a.i./ha, DPX-YT669 (picoxystrobin) @ 150 g a.i./ha, DPX-Q8X63-001 (penthiopyrad + picoxystrobin) at 200, 300, and 400 g a.i./ha, and Lance at 290 g a.i./ha, plus a nontreated control. Ten plants per plot were selected at random and assessed for ascochyta blight severity on whole plants (foliage and stems) on 18 August, and 03 and 17 September. Crop tolerance (% foliar damage/injury) was assessed on 03 and 17 September; no injury was observed in any plot. The trial was not harvested, as only a few of the plants had produced any mature seed before they were killed by frost. Analysis of variance (General Linear Model Procedure of SAS) was conducted to assess the impact of treatments, with means separation using Tukey's test. Differences are significant at $P = 0.05$ unless otherwise stated.

RESULTS

2009: Soil conditions in spring were so dry that most of the seedlings did not emerge until irrigation was applied. This resulted in uneven stands throughout the season and increased variability in assessments of foliar disease. Ascochyta blight severity was very low when the first fungicide treatment was applied (Table 7). This low level of ascochyta blight indicates that conditions early in the growing season were not conducive for disease development. At the 2nd rating date, severity had increased slightly and several of the fungicides limited disease increase. At the 3rd rating date, all of the fungicides reduced severity relative to the control, and there were small (but likely not biologically meaningful) differences among the fungicide treatments. Late germination, uneven seedling establishment and an unusually cool growing season prevented this late maturing chickpea cultivar from completing development and producing seed.

Table 7. Impact of two applications of foliar fungicide on severity of ascochyta blight (0–11 scale) on chickpea cv. CDC Frontier at Saskatoon in 2009.

Fungicide treatment	Rate (g a.i./ha)	Blight severity (0–11 scale)		
		Aug. 11	Aug. 22	Sept. 04
Nontreated control	---	1.0 a	2.2 a	3.3 a
Bravo + Quadris	1500/124	1.1 a	1.6 abcd	2.0 e
Lance	290	1.1 a	1.5 bcd	1.9 e
Penthiopryad EC	150	1.2 a	1.6 abcd	2.4 bc
	250	1.2 a	1.5 abcd	2.3 bc
DPX-Q8X63-001	200	1.2 a	1.7 abc	2.5 b
	300	1.2 a	1.4 bcd	2.3 cd
	400	1.2 a	1.3 bcd	2.1 de
DPX-YT669	150	0.9 a	1.0 cd	2.3 bc
DPX-Q8X63-002	300	2.2 a	1.0 d	2.0 e

(*a*–*e*) Means in a column followed by the same letter do not differ at $P = 0.05$ based on the Waller-Duncan K-ratio T test.

2010: Early in July, ascochyta blight severity was very low but the disease was present in almost every plot, indicating that low levels of natural inoculum of *A. rabiei* were present at the site. Chickpea residue that was heavily infested with ascochyta blight was applied evenly across the plot area to increase disease pressure in the trial. At the first rating date (prior to application of the foliar fungicides), severity was low (mean rating = 1.7) and there were no differences among the treatments. At the second date, severity in the control had increased slightly but the disease had not progressed in plots treated with foliar fungicide. At the final rating date, blight severity was higher in the control than in

the foliar fungicide treatments. The lowest rate of penthiopyrad + picoxystrobin (200 g ai) provided only an intermediate level of disease reduction relative to the higher rates (Table 8). There was no evidence of phytotoxicity for any of the products assessed.

Table 8. Effect of foliar fungicides on whole plant disease ratings on chickpea cvs. CDC Xena and CDC Frontier inoculated with *Ascochyta rabiei* at Saskatoon in 2010.

Foliar fungicide treatment	Blight severity (0–11 scale)		
	Aug. 18	Sept. 3	Sept. 17
Untreated control	1.6 a	2.6 a	3.4 a
Lance @ 290 g ai/ha	1.7 a	1.6 b	1.9 c
Picoxystrobin SC @ 150 g ai/ha	1.9 a	1.6 b	2.2 bc
Penthiopyrad EC @ 200 g ai/ha	1.8 a	1.7 b	2.2 c
Penthiopyrad + picoxystrobin @ 200 g ai/ha	1.8 a	1.4 b	2.6 b
Penthiopyrad + picoxystrobin @ 300 g ai/ha	1.6 a	1.4 b	2.1 c
Penthiopyrad + picoxystrobin @ 400 g ai/ha	1.6 a	1.6 b	2.0 c

(*a-c*) Means in a column followed by the same letter do not differ based on the Tukey's test at $P = 0.05$.

DISCUSSION

Across all years and rates, application of penthiopryad consistently reduced the severity of ascochyta blight and there was no evidence of phytotoxicity. Both formulations of penthiopryad were effective.

In 2007, the severity of ascochyta blight was low but all of the fungicides except Manzate increased seed yield compared to the control. In 2008, disease pressure was low to moderate until late in the season. Efficacy of penthiopryad increased with increasing rate up to 250 gm, with the best treatment yielding more than 200% of the control in 2008. This indicates that two spray applications provided good control of blight on this highly susceptible cultivar.

In 2009, disease pressure was low due to extreme early-season drought. Application of penthiopryad and DPX fungicides consistently reduced the severity of ascochyta blight and there was no evidence of phytotoxicity at any rate.In 2010, seeding was delayed by saturated soil conditions until mid-June, which is much later than recommended for this long-season crop. More rain after seeding resulted in flooding and stand loss. The plots that did survive were thin and uneven throughout the season. The crop canopy in these thin stands did not close, which slowed blight development. As a result, only a single application of fungicide was required to maintain good control of foliar disease. Cool conditions during the growing season slowed crop development, and the trial was killed

by frost before seed could mature. A single application of penthiopyrad and/or picoxystrobin reduced ascochyta blight severity and provided effective disease control, similar to Lance, which was selected as the commercial standard. The only exception was the lowest rate of penthiopyrad + picoxystrobin (200 g ai), which was less effective than the higher rates or penthiopyrad alone.

C.2 Other actives

Several other novel actives have been examined in the course of the project. Several of these were included as treatments in existing trials with penthiopyrad, but have been separated out in this presentation to highlight that these represent distinct products from rival companies.

MATERIALS AND METHODS

2009: Agress (90% oxysilver nitrate) and IOT-SP (silver periodate, Innovotech Inc., Edmonton, AB); Bravo 500 (chlorothalonil 50%) and Quadris (azoxystrobin, 250g/L; Syngenta Crop Protection Canada Inc., Guelph, ON); Lance 70% DF (boscalid, BASF Canada Inc., Toronto, ON); Proline (prothioconazole, Bayer CropScience Inc., Calgary, AB).

A trial to assess the impact of foliar fungicide application on ascochyta blight severity and seed yield of chickpea cv. CDC Frontier was conducted on a Sutherland clay-loam soil at the AAFC Research Farm at Saskatoon in 2009. CDC Frontier is a kabuli chickpea with a fern leaf type, late maturity and has fair ascochyta blight resistance (Saskatchewan Agriculture and Food, 2009). The plot area had been seeded to barley the previous year.

The layout and management of the trials were as described previously. The plots were direct seeded on 26 May, 2009 at about 45 plants per m^2 using a double-disc drill seeder. Germination was delayed and irrigation was required as a result of drought conditions in spring. Overhead irrigation was applied on 12, 15, and 16 June (2.4, 1.5, and 1.0 cm of water applied, respectively). Chickpea residue that had been collected in 2008 from a crop that was heavily infested with ascochyta blight was applied evenly to the plot area on July 28 to initiate an epidemic of ascochyta blight.

There were seven fungicide treatments in the trial, each applied at early bloom (10 August) and late bloom (25 August) stage: Agress at 17.5 g a.i./ha and 35 g a.i./ha; IOT-SP at 17.5 g a.i./ha and 35 g a.i./ha; Lance at 290 g a.i./ha; Proline at 200 g a.i./ha; Bravo 500 at 1.5 kg a.i./ha at early bloom and Quadris at 120 g a.i./ha at late bloom, and a nontreated control.

Ten plants per plot were selected at random and assessed for ascochyta blight severity on whole plants on 11, 22 August and 04 September. Crop tolerance was assessed on August 22 and September 04; no injury was observed in any plot. The trial was not harvested because the plants were killed by frost before the seed matured.

2010: Biological control agent (name withheld by request)); PROLINE 480 SC (prothioconazole, Bayer CropScience Inc., Calgary, AB); LANCE 70% DF (boscalid, BASF Canada Inc., Toronto, ON).

A trial to assess the impact of foliar fungicide application on ascochyta blight severity and seed yield of chickpea cvs. CDC Frontier and CDC Xena was conducted on a Sutherland clay-loam soil at the AAFC Research Farm at Saskatoon in 2010. CDC Frontier is a kabuli cultivar with fern leaf type, late maturity and has fair ascochyta blight resistance. CDC Xena is a kabuli cultivar with unifoliate leaf type, late maturity and very poor ascochyta blight resistance (Saskatchewan Agriculture and Food, 2010).

Seeding was delayed by heavy rain and cool conditions. Several early plantings were drowned out, and even the last seeding date did not establish well. As a result, the best plots from each of two adjacent trials were combined into one test with two reps of CDC Frontier and two reps of CDC Xena.

The plots were direct seeded at about 45 plants per m^2 using a double-disc drill seeder on 15–16 June. The studies were laid out as a RCBD with four replications, with plot layout and maintenance as described previously. Crop residue collected in 2009 from a chickpea field that was heavily infested with ascochyta blight was applied evenly to the plot area on 28 July to initiate the blight epidemic.

Four foliar fungicide treatments were applied on August 19 at the mid-bloom stage: the biocontrol agent, Proline @ 0.42 L/ha, Serenade (5 L) + Proline (0.42 L), and Lance @ 290 g a.i./ha, plus an untreated control. The treatments were applied with a CO_2 backpack sprayer (Model GS, R&D Sprayers, Inc.) equipped with an aluminum spray boom with four XR TeeJet 8002 VS nozzles spaced 48 cm apart, calibrated to deliver 250 L/ha at 240 kPa. Ten plants per plot were assessed for ascochyta blight severity on 18 August, 03 and 17 September. Crop tolerance (% foliar damage/injury) was assessed on 03 and 17 September; no injury was observed in any plot. The trial was not harvested, as only a few of the plants had produced any mature seed before they were killed by frost.

RESULTS

2009: Soil conditions in spring of 2009 were so dry that most of the seedlings did not emerge until irrigation was applied. This resulted in uneven stands throughout the season and increased variability in assessments of foliar disease. Ascochyta blight severity was very low when the first fungicide treatment was applied (Table 9). This low level of ascochyta blight indicates that conditions early in the growing season were not conducive for disease development. At the 2nd rating date, severity had increased slightly and several of the fungicides limited disease increase. At the 3rd rating date, all of the fungicides had reduced severity relative to the control, and there were small (but likely not biologically meaningful) differences among the fungicide treatments. Late germination, uneven seedling establishment and an unusually cool growing season prevented this late maturing chickpea cultivar from completing development and producing seed.

Table 9. Impact of two applications of foliar fungicide on severity of ascochyta blight (0–11 scale) on chickpea cv. CDC Frontier at Saskatoon in 2009.

Fungicide treatment	Rate (g a.i./ha)	Blight severity (0–11 scale)		
		Aug. 11	Aug. 22	Sept. 04
Nontreated control	---	1.0 a	2.2 a	3.3 a
Lance	290	1.1 a	1.5 ab	1.9 cd
Proline	200	1.1 a	1.1 b	1.8 d
Bravo + Quadris	1500/124	1.1 a	1.6 ab	2.0 c
Agress	17.5	0.9 a	1.8 ab	2.3 b
	35	1.1 a	1.3 b	1.9 cd
IOT-SP	17.5	1.0 a	1.5 ab	2.2 b
	35	1.1 a	1.8 ab	2.0 c

(*a–d*) Means in a column followed by the same letter do not differ based on the Waller-Duncan K-ratio T test at $P = 0.05$.

2010: Early in July, ascochyta blight severity was very low but the disease was present in almost every plot, indicating that low levels of natural inoculum of *A. rabiei* were present at the site. Chickpea residue that was heavily infested with ascochyta blight was applied evenly across the plot area to increase disease pressure in the trial. At the first rating date (prior to fungicide application), severity was low (mean rating = 1.6) and there were no differences among treatments (Table 10). At the second date, severity in the control had increased slightly but the disease had not progressed in plots treated with foliar fungicide. At the final rating date, blight severity was higher in the control than in the foliar treatments, and there were no differences among the foliar treatments (Table 10).

Table 10. Effect of foliar fungicides on whole plant disease ratings on chickpea cvs. CDC Xena and CDC Frontier inoculated with *Ascochyta rabiei* at Saskatoon in 2010.

Fungicide treatment	Rate (ha^{-1})	Blight severity (0–11 scale)		
		Aug. 18	Sept. 03	Sept. 17
Nontreated control	---	1.6 a	2.6 a	3.4 a
Biocontrol agent (BA)	5 L	1.6 a	1.6 b	1.9 b
BA + Proline	5 L + 0.42 L	1.5 a	1.5 b	1.9 b
Lance	294 g ai	1.7 a	1.6 b	1.9 b

(*a-b*) Means in a column followed by the same letter do not differ based on the Tukey's test at $P = 0.05$.

DISCUSSION

Disease pressure was low in 2009. Application of Agress and IOT-SP fungicides consistently reduced the severity of ascochyta blight. There was no evidence of phytotoxicity at any rate.

In 2010, seeding was delayed by saturated soil conditions until mid-June, which is much later than recommended for this long-season crop. More rain after seeding resulted in flooding and stand loss. The plots that did survive were thin and uneven throughout the season. The crop canopy in these thin stands did not close, which slowed the development of ascochyta blight. As a result, only a single application of fungicide was required to maintain good control of foliar disease. Cool conditions during the growing season slowed crop development, and the trial was killed by frost before seed could mature. A single application of the biocontrol agent (BA) or BA + Proline reduced ascochyta blight severity and provided effective disease control, similar to Lance, which was selected as the commercial standard. There was no evidence of phytotoxicity for any of the products assessed.

D. PENTHIOPYRAD, CHLOROTHALONIL AND MANCOZEB

The objectives of this study were to: i) estimate the baseline sensitivity of *A. rabiei* to penthiopyrad, a new SDHI fungicide, and determine if cross-resistance to penthiopyrad and boscalid is present in the pathogen population, and ii) assess sensitivity of *A. rabiei* to the multi-site inhibitor fungicides chlorothalonil and mancozeb to determine if there has been a shift to insensitivity in pathogen population.

MATERIAL AND METHODS

Isolates of *A. rabiei*: To collect the isolates, two plants with ascochyta blight symptoms were collected at each of five sites per field from 12 commercial fields in Saskatchewan in 2008 and 8 fields in 2009. The samples were returned to the laboratory for isolation using the methods described in the previous chapter. The purpose was to produce at least 10 isolates per field, each from a different plant, to represent the pathogen population in the field. Fifty isolates of *A. rabiei* that had never been exposed to penthiopyrad were obtained from infected chickpea plants collected in 2008 for assessment of baseline sensitivity. An additional 32 isolates of *A. rabiei* collected in 2008 and 47 isolates collected in 2009, which had never been exposed to penthiopyrad, were used to evaluate a discriminatory dose. Another 19 isolates of *A. rabiei* collected in 2004 in Saskatchewan prior to the commercial introduction of boscalid were used to determine the correlation in response between these two actives.

The isolates of *A. rabiei* collected from infected chickpea plants in Saskatchewan in 2004, 2007, 2008 and 2009 are all assumed to have had prior exposure to the multi-site inhibitor fungicides, because these fungicides are used on a large portion of the acreage every year (Gossen, personel communication). Therefore, isolates collected in 2007 were selected at random for assessment of sensitivity to chlorothalonil and mancozeb. Three isolates were used in a preliminary assessment of chlorothalonil and mancozeb to select the range of concentration for use in subsequent assessment of radial growth. The radial growth of 15 isolates was assessed to both fungicides to calculate EC_{50} values, and 11 additional isolates were assessed using the discriminatory doses selected for chlorothalonil and mancozeb. Another 21 isolates of *A. rabiei* collected in 2004, 2008, and 2009 were subsequently assessed at the discriminatory dose for chlorothalonil. Thus, a total of 50 isolates were tested for sensitivity to chlorothalonil and 27 isolates were tested for sensitivity to mancozeb.

Sensitivity to penthiopyrad and boscalid: Fungicide sensitivity was evaluated *in vitro*, based on radial growth on PDA amended with technical grade active ingredient of penthiopyrad (DuPont Canada, Mississauga, ON), and boscalid (BASF, Canada Inc., Mississauga, ON). Stock solutions of the technical grade active ingredient of penthiopyrad and boscalid were prepared at a concentration of 100 mg mL^{-1} in acetone. Each fungicide was added just prior to pouring the media into Petri dishes. Control treatments were amended only with acetone (0.1% by volume). There was one dish per replicate, three replicates per treatment arranged in a completely randomized design, and each experiment was repeated.

For baseline assessments, the sensitivity of *A. rabiei* to penthiopryad was assessed using 50 isolates collected in 2008 in Saskatchewan, Canada. The effective concentration to inhibit radial growth of mycelium by 50% (EC_{50}) was estimated for each isolate on PDA amended with technical grade penthiopryad at 0.01, 0.1, and 1 µg/mL with three replicates per treatment. EC_{50} values ranged from 0.002 to 0.30 µg/mL with a mean of 0.10 µg/mL.

A discriminatory dose of 0.3 µg/mL was selected for assessment of 32 additional isolates collected in 2008 and 47 isolates collected in 2009 (never exposed to penthiopryad); 12 of the 79 isolates exhibited < 50% growth inhibition. For assessments of cross-resistance, 19 isolates were assessed on PDA amended with technical grade active penthiopyrad at 0, 0.01, 0.1, and 1.0 µg mL^{-1} or 0.05, 0.5, and 5.0 µg mL^{-1} boscalid. All experiments were prepared using the methods described on the previous section. The percent reduction of radial growth for each isolate in each replicate was used in PROC PROBIT in SAS 9.1 (SAS Institute, Cary, NC) against the log_{10} of the fungicide concentration to calculate an EC_{50} value.

Sensitivity to chlorothalonil and mancozeb: Fungicide sensitivity was evaluated *in vitro*, based on radial growth on PDA amended with formulated product of chlorothalonil (Bravo 500 EC; Syngenta Crop Protection Canada Inc., Guelph, ON), and mancozeb (Dithane DG 75%; Dow AgroScience Canada Inc., Calgary, AB). Three isolates collected in 2007 in Saskatchewan were selected for the preliminary assessment to select the optimum range of concentrations to both fungicides. Stock solutions of chlorothalonil and mancozeb were prepared at a concentration of 100 mg ml^{-1} in water. PDA was amended with formulated product to achieve final concentrations of 0, 0.1, 1, 10, 100, and 1000 µg mL^{-1} for both chlorothalonil and mancozeb for a preliminary assessment based on reduction of radial growth as described previously. Control treatments were unamended. Based on the results of this preliminary assessment, 15 isolates were assessed at concentrations of 0, 10, 25, 75, and 325 µg mL^{-1} and EC_{50} values were calculated as described previously. An additional 11 isolates collected in 2007 in Saskatchewan were assessed at a discriminatory dose of 250 µg mL^{-1} chlorothalonil, and 9 of these 11 isolates were assessed at a discriminatory dose of 100 µg mL^{-1} mancozeb. Finally, an additional 21 isolates of *A. rabiei* collected in 2004, 2008, and 2009 were assessed at the discriminatory dose of 250 µg mL^{-1} chlorothalonil. All experiments were prepared using the methods described on the previous chapter.

Statistical analyses: In each experiment, the two repetitions did not differ at $P = 0.05$ and so the data were pooled for analysis. A correlation analysis of the EC_{50} values of penthiopyrad and boscalid for 19 isolates was conducted using PROC CORR in SAS to determine if there was a relationship between the reactions of individual isolates to these two closely related actives. The percent inhibition of radial growth distributions for each isolate was tested for normality using Shapiro-Wilk test (PROC UNIVARIATE).

RESULTS

Sensitivity to penthiopyrad and boscalid: The 50 baseline isolates collected in 2008 had EC_{50} values that ranged from 0.002 to 0.30 μg mL^{-1}, with a mean of 0.1 μg mL^{-1} (Fig. 11). A discriminatory dose of 0.3 μg mL^{-1} was selected for penthiopyrad for screening of a wider population and used to assess 32 isolates collected in 2008 and 47 isolates collected in 2009; 12 of the 79 isolates (15%) exhibited less than 50% inhibition of radial growth (Fig. 12).

When the response to penthiopyrad and boscalid was compared in isolates collected in 2004, each of the 19 isolates was sensitive to both actives. EC_{50} values for radial growth ranged from 0.02 to 0.25 μg mL^{-1} with a mean of 0.08 μg mL^{-1} for penthiopyrad, and 0.03 to 0.13 μg mL^{-1} with a mean of 0.05 μg mL^{-1} for boscalid. The mean EC_{50} values for both penthiopyrad and boscalid were heavily skewed to the left, which means that most of the isolates that were strongly inhibited at low concentrations. There was no correlation ($r = 0.07$, $P = 0.77$) between EC_{50} values of penthiopyrad and boscalid (Fig. 13).

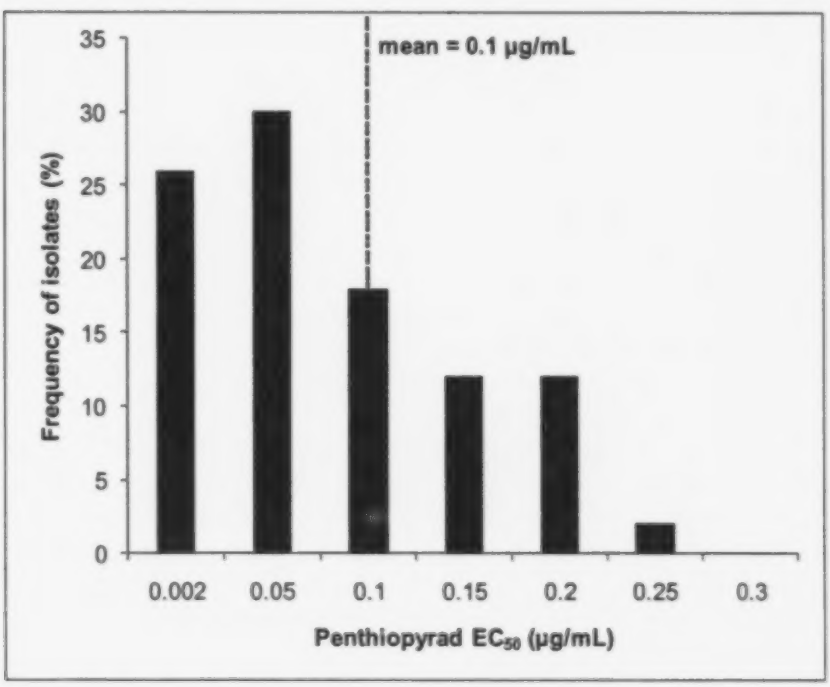


Fig. 11. Baseline sensitivity of *Ascochyta rabiei* to penthiopyrad based on a radial growth assay of 50 isolates collected in Saskatchewan in 2008.

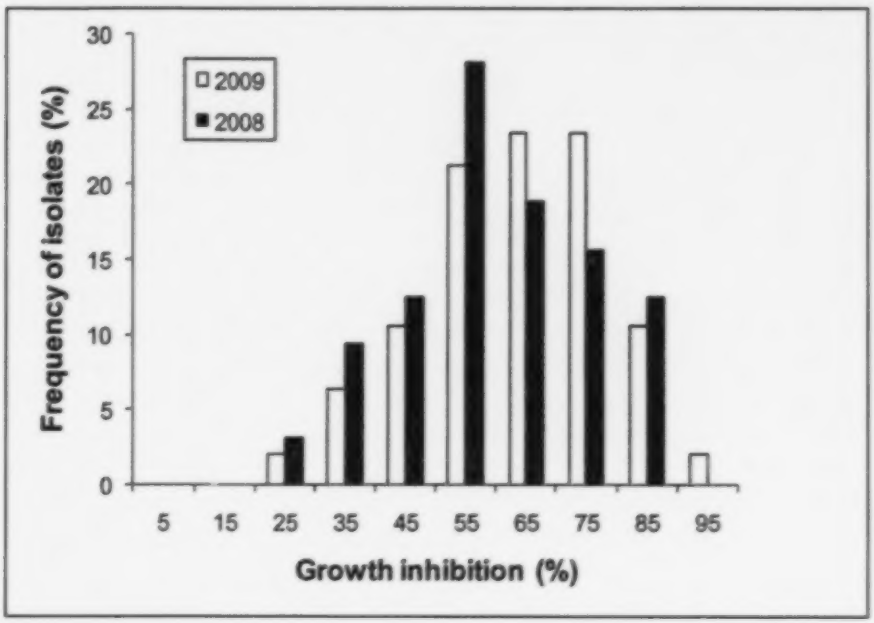


Fig. 12. Growth inhibition (%) of *Ascochyta rabiei* isolates collected in Saskatchewan in 2008 (n = 32) and 2009 (n = 47) at a discriminatory dose of 0.3 μg mL^{-1} of penthiopyrad.

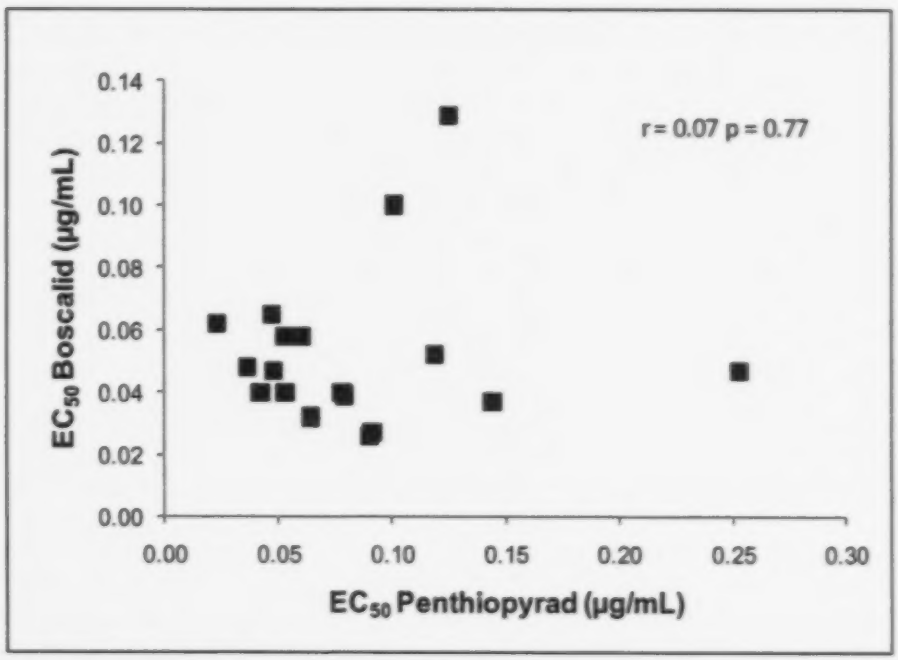


Fig. 13. Comparison of sensitivity to penthiopyrad and boscalid of 19 isolates of *A. rabiei* collected in 2004 (never exposed to either fungicide) based on radial growth.

Sensitivity to chlorothalonil and mancozeb: The EC_{50} values for the three isolates in the preliminary assessment ranged from 19 to 166 μg mL^{-1} for chlorothalonil and from 18 to 46 μg mL^{-1} for mancozeb. In the main assessment of 15 isolates, there was a moderate range of EC_{50} values for chlorothalonil, from 16 to 76 μg mL^{-1}, with a mean of 58 μg mL^{-1} (Fig. 14). In addition, one isolate showed a high EC_{50} value of 206 μg mL^{-1}, which was outside of the frequency distribution of chlorothalonil. The $\log_{10}$ EC_{50} values of chlorothalonil were not normally distributed ($P = 0.043$). However, the distribution of the isolates was skewed towards lower EC_{50} values which mean most of the isolates were inhibited at 75 μg mL^{-1}.

Of the 32 isolates assessed at the discriminatory dose of 250 μg mL^{-1} chlorothalonil, 94% of the isolates showed more than 50% inhibition (Figure 15) and growth inhibition at the discriminatory dose was normally distributed for isolates collected in 2007 ($P = 0.47$). However, one isolate exhibited less than 50% growth inhibition and another isolate exhibited 50% growth inhibition to chlorothalonil.

For mancozeb, EC_{50} values ranged from 16 to 57 μg mL^{-1}, with a mean of 37 μg mL^{-1} (Fig. 16). All nine of the isolates assessed at the discriminatory dose of 100 μg mL^{-1} mancozeb were inhibited at this dose (Fig. 17). The $\log_{10}$ EC_{50} values of mancozeb were normally distributed ($P = 0.10$). All isolates were inhibited at 60 μg mL^{-1}.

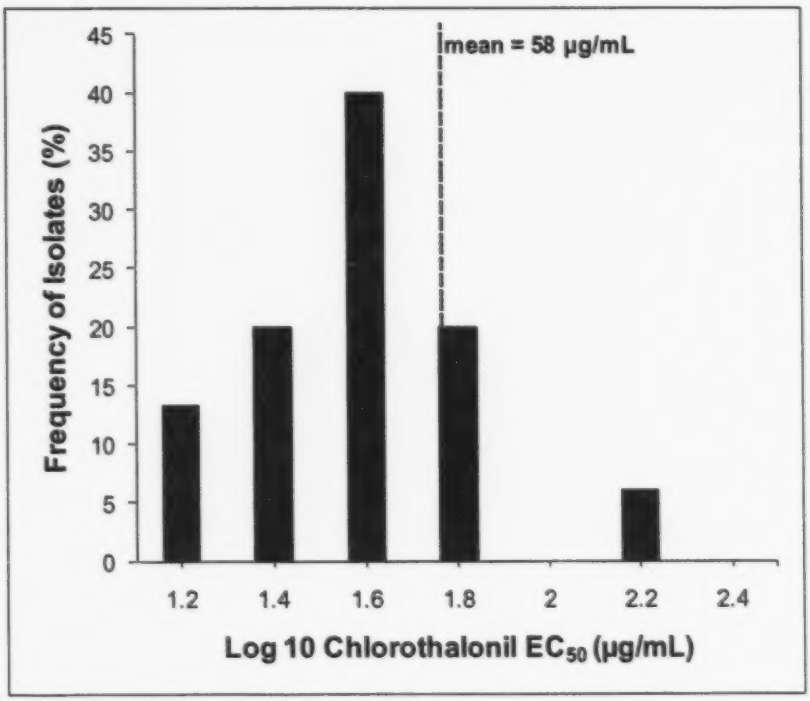


Fig. 14. Sensitivity to chlorothalonil of 15 isolates of *Ascochyta rabiei* collected in 2007 in a radial growth assay.

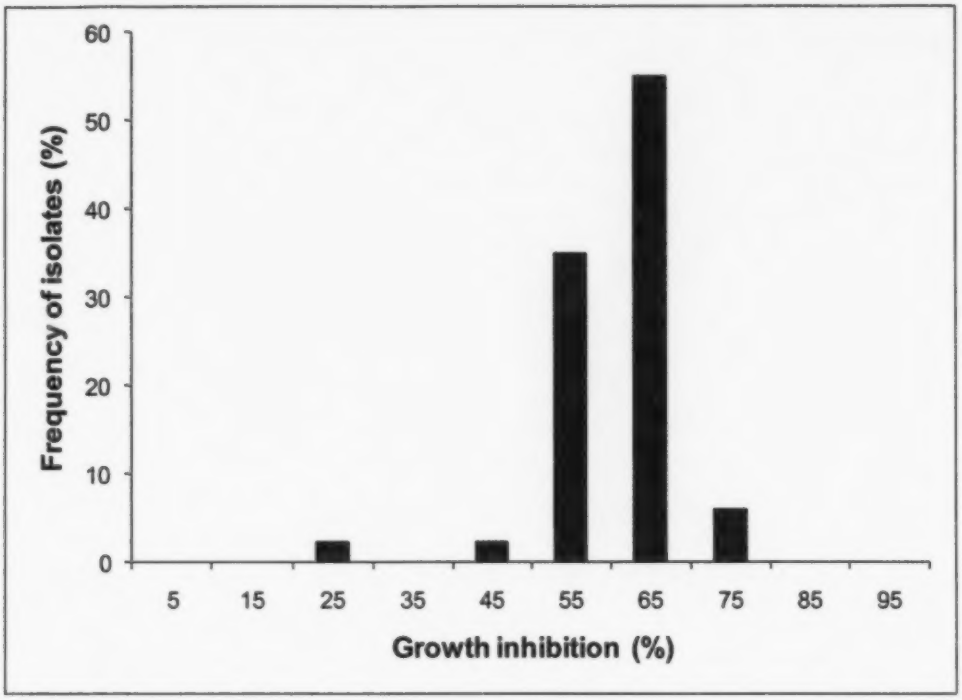


Fig. 15. Frequency distribution of 32 isolates of *Ascochyta rabiei* combined across years, based on growth inhibition at 250 µg mL^{-1} of chlorothalonil.

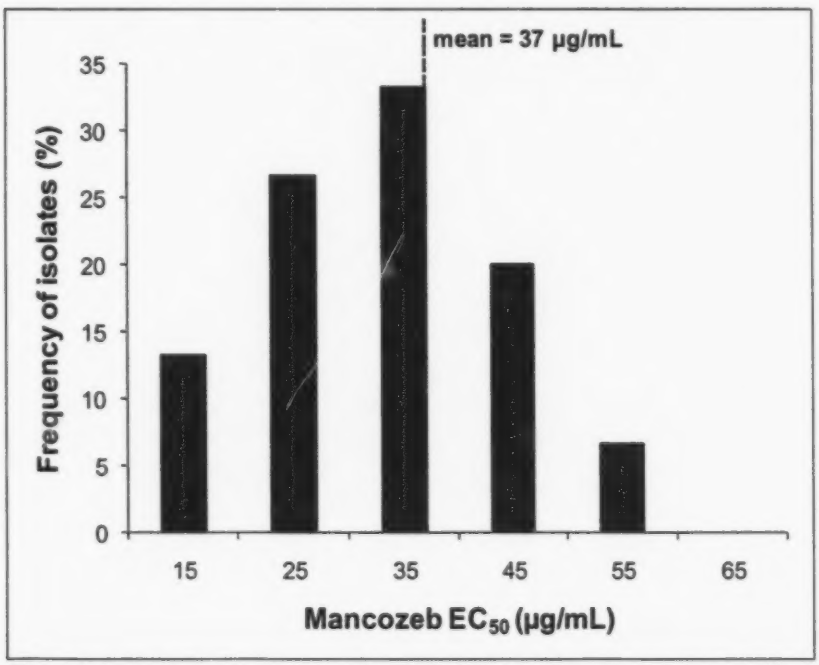


Fig. 16. Sensitivity to mancozeb of 15 isolates *Ascochyta rabiei* collected in 2007 based on inhibition of radial growth.

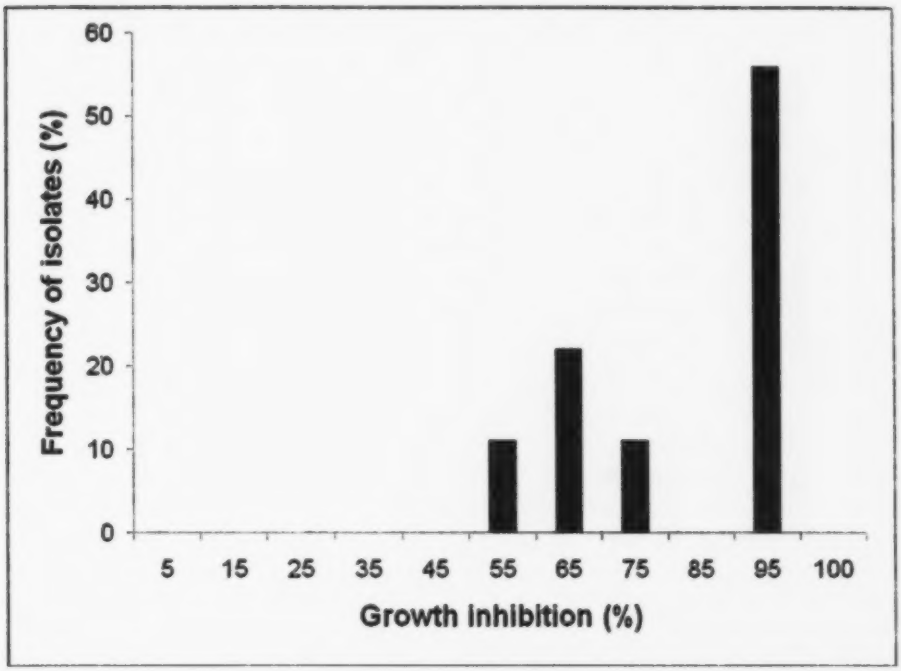


Fig. 17. Sensitivity to mancozeb of nine isolates of *Ascochyta rabiei* collected in 2007 based on inhibition of radial growth at a discriminatory dose.

DISCUSSION

No insensitivity to penthiopyrad or boscalid in *A. rabiei* was identified in the present study. The baseline sensitivity for conidial germination of *A. rabiei* isolates to boscalid in the USA ranged from EC_{50} values of 0.02 to 0.49 μg mL^{-1}, with a mean of 0.19 μg mL^{-1} (Wise et al. 2008). Although this mean EC_{50} value is numerically higher than the estimate obtained in the present study (0.05 μg mL^{-1}), the range of baseline EC_{50} values to boscalid for 19 isolates of *A. rabiei* in the current study (0.03-0.13 μg mL^{-1} based on radial growth assessments) lies within the range reported in the previous study.

A discriminatory dose of 0.3 μg mL^{-1} penthiopyrad was selected to evaluate a wider population of isolates for sensitivity to penthiopyrad. This discriminatory dose was selected to lie just above the maximum EC_{50} value observed in the baseline population, to identify small changes in sensitivity. All of the *A. rabiei* isolates assessed were sensitive to both boscalid and penthiopyrad. The 19 isolates from 2004 were collected prior to the commercial introduction of boscalid, while the isolates collected in 2007, 2008, and 2009 may have been exposed to boscalid; 15% of the isolates collected in 2008 and 2009 exhibited less than 50% inhibition to 0.3 μg mL^{-1} penthiopyrad. This indicates that a substantial proportion of the pathogen population in 2008 and 2009 were slightly less sensitive than isolates with no exposure to SDHIs. A positive correlation between sensitivity to penthiopyrad and boscalid has recently been reported in the closely related pathogen, *D. bryoniae* (Thomas et al, 2010), so there is a high potential for cross-

resistance between these two actives for *A. rabiei*. Taken together, this result indicates that exposure to boscalid may have already changed the sensitivity of the pathogen population to both of these SDHI fungicides.

The single-site mode of action of SDHI fungicides is an important risk factor for development of insensitivity in fungal pathogens. Insensitivity to boscalid has been reported in field isolates of other fungal pathogens and *A. rabiei* is considered a high-risk pathogen for developing insensitivity to fungicides. Establishing the baseline sensitivity of *A. rabiei* to SDHI fungicides will contribute to future monitoring studies to detect early shifts in the sensitivity of the pathogen, in advance of breakdown of control in the field. Based on the reaction of other pathogens to boscalid, we expect that gradual shift towards reduced sensitivity to SDHIs will occur in *A. rabiei* with repeated use. Also, cross-resistance between penthiopyrad and boscalid is expected. We conclude that *A. rabiei* is at risk of developing insensitivity to these SDHI fungicides under high fungicide selection pressure. For future monitoring, we suggest using a higher discriminatory dose (e.g., 1.0 $\mu g\ mL^{-1}$) to detect larger changes in sensitivity to SDHI fungicides. We also suggest that the correlation between boscalid and penthiopyrad be reassessed using numerous isolates that have been exposed to SDHIs over the next few years.

Variability in the sensitivity of *A. rabiei* isolates to the multi-site inhibitor fungicides chlorothalonil and mancozeb has been reported in western Canada (Chang et al. 2007). In this study, the sensitivity of *A. rabiei* isolates to chlorothalonil and mancozeb was determined using a radial growth assay. All 27 isolates of *A. rabiei* isolates were sensitive and consistent in their response to mancozeb.

The EC_{50} values for chlorothalonil for the 15 isolates collected in Saskatchewan in 2007 in the initial assessment ranged from 16 to 76 $\mu g\ mL^{-1}$, except for one isolate with an EC_{50} value of 206 $\mu g\ mL^{-1}$. In addition, two of the isolates assessed at a discriminatory dose of 250 $\mu g\ mL^{-1}$ exhibited low inhibition of radial growth.

The observation that some isolates are relatively insensitive to chlorothalonil is interesting and requires additional assessment, but may not result in loss of efficacy in the field. Although both radial growth and conidial germination assays have been used for assessment of sensitivity to multi-site fungicides and the protectant fungicides have been shown to affect on all stages of fungal development (Bruck et al., 1981; Daay & Platt, 2002), these fungicides have a much more powerful impact on conidial germination than radial growth. A previous study showed that chlorothalonil inhibited conidial germination of *A. rabiei* at low concentrations but did not affect radial growth (Demirci et al. 2003). More importantly, no breakdown in efficacy of chlorothalonil has been reported on chickpea under field conditions. In the present study, a radial growth assay with a single discriminatory dose was selected for assessment of sensitivity to chlorothalonil because it provides a relatively simple and efficient test. However, future studies to assess sensitivity to chlorothalonil should focus on inhibition of conidial germination, because this factor is likely to be more closely correlated with efficacy against ascochyta blight in the field than radial growth assessments.

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TECHNOLOGY TRANSFER

The most important component of the technology transfer that was conducted for this project was actually performed by Sask. Agric. staff and the companies that produce and distribute strobilurin fungicides. They took the message about potential breakdown in control with strobilurins on chickpea to producers in a rapid and effective manner. The presentations that were made by our group as part of this program are listed under Technology Transfer in the publications section of this report.

PERFORMANCE INDICATORS

Impact

Prior to the 2008 field season, companies that sold strobilurin fungicides, extension professionals, and growers were advised that a dramatic shift in sensitivity to strobilurin fungicides had occurred. As a result, fungicide recommendations were changed for the 2008 field season, at both the extension level and by retailers. That change, coupled with dry conditions across much of the chickpea production area in July, kept the disease in check in most fields. This carried over into the 2009 season, where dry conditions in spring reduced disease pressure in most areas, which gave growers time to adjust their fungicide application plans. Again, levels of ascochyta blight were low throughout the region. In 2010, high precipitation across the chickpea production area provided conditions that were highly conducive to ascochyta blight. However, disease levels in most fields were low to moderate, indicating that producers had adjusted their fungicide usage patterns and were able to manage ascochyta blight even though one of the most important groups of fungicide for control of this pathogen had been lost.

Benefits

By providing information on the breakdown in resistance to strobilurin resistance to the life science industry and producers in a timely manner, devastating losses from ascochyta blight from loss in efficacy of strobilurin fungicides were avoided. This resulted in sustained revenue to producers and huge benefits to the manufacturers and retailers of strobilurin products. Although retailers did not sell as much product, they also did not have to deal with litigation over an unexpected breakdown of efficacy.

Human resources

A graduate student (Nael Thaher) was recruited and joined the project in September 2008, based in the University of Guelph. He spent the summer of 2009 working out of the AAFC Research Centre at Saskatoon, where he was involved in field studies and disease surveys. He completed his thesis and the other requirements for an MSc degree from the University of Guelph in January 2011.

The techniques and expertise that have been developed as part of this project are being used to assess fungicide sensitivity in other important pulse pathogens. For example, an international collaboration on fungicide sensitivity in *Mycosphaerella pinodes* (an important pathogen of field pea) has been established (recent results published as Bowness, R., Chang, K.F., Gossen, B.D., Goswami, R.S., Hwang, S.F., Willenborg, C., and Strelkov, S.E. 2010. Baseline sensitivity of *Mycosphaerella pinodes* to pyraclostrobin fungicide. Pg. 3–4 in Proc. Can. Pulse Research Workshop (Oral

Presentations), Calgary, AB, Nov. 3–5, 2010; Delgado, J.A., Stoppler, T., Gossen, B.D., Chang, K.F., Dugan, F., Markell, S., and Goswami, R.S. 2010. Sensitivity of *Ascochyta pinodes* populations to prothioconazole. Pg. 11–12 in Proc. Can. Pulse Research Workshop (Posters), Calgary, AB, Nov. 3–5, 2010).

The expertise from this project is also being used to develop recommendations to prairie producers for crops such as canola (Kutcher, H.R., Turkington, T.K., Gossen, B.D., Brandt, S.A., Dokken-Bouchard, F., and Strelkov, S. 2011. Canola disease and fungicide strategies based on crop rotation. Invited presentation and report abstract, Strategies for Successful Farming, Viterra, on 01 March, 2011 at Saskatoon and 02 March, 2011 in Regina, SK.; Kutcher, H.R., Eynck, C., Turkington, T.K., Gossen, B.D., and Brandt, S.A. 2010; Pest implications of intensive canola rotations. Invited presentation, SK Plant Disease Comm., Dec. 10, 2010 and as an update for staff of SK Agric. and Food on Dec. 16, 2010, and Kutcher, H.R., Eynck, C., Turkington, T.K., Gossen, B.D., and Brandt, S.A. 2010. Implications of intensive canola rotations. Invited presentation and proceedings paper, Can. Weed Sci. Soc., Nov. 17, 2010).

New technology

A protocol for assessing sensitivity to strobilurins that is suitable for use in diagnostic and extension laboratories was developed. Several new actives with efficacy against *A. rabiei* were assessed, and are at various stages in the testing and registration process. Vertisan (a.i. penthiopyrad) is expected to be available to producers in 2011 or early 2012. In addition, studies of the baseline sensitivity for some of the newer fungicide actives used to control ascochyta blight were conducted. No insensitive isolates were identified, but the studies will provide as a baseline for future monitoring of changes in sensitivity to these actives. Finally, testing of the old multi-site actives has raised questions about potential changes in the efficacy of chlorothalonil that need to be addressed quite quickly. Alternative fungicides for management of ascochyta blight were tested and effective alternatives are coming onto the market.

PUBLICATIONS RELATED TO THE PROJECT

Refereed publications

Gossen, B.D., Hwang, S.F., Thaher, N.H., Ahmed, H.U., Chang, K.F., Turnbull, G.D. and McDonald, M.R. 2011. Shift to insensitivity to QoI fungicides in *Ascochyta rabiei* in western Canada. Can. J. Plant Pathol. xx: xx–xx (submitted).

Other Scientific

Thaher, N.H., Gossen, B.D., and McDonald, M.R. 2011. Comparison of methods to assess the sensitivity of *Ascochyta rabiei* to strobilurin fungicides. Can. J. Plant Pathol: 32: xxx (Abstract, In press).

2010

Gossen, B.D., Thaher, N.T., and McDonald, M.R. 2010. Sensitivity of *Ascochyta rabiei* to strobilurins and other foliar fungicides in western Canada. Pg. 5 in Proc. Can. Pulse Research Workshop (Oral Presentations), Calgary, AB, Nov. 3–5, 2010.

Thaher, N.H., Gossen, B.D., and McDonald, M.R. 2010. Developing a method to assess insensitivity to pyraclostrobin fungicide in *Ascochyta rabiei*. Can. J. Plant Pathol: 32: 410. (also pg. 13−14 *in* Proc. Plant Pathol. Soc. Alberta, October 26−28, 2009, Canmore, AB.).

Thaher, N.H., Gossen, B.D., and McDonald, M.R. 2010. Baseline sensitivity of *Ascochyta rabiei* to penthiopryad, a new SDHI fungicide. Phytopathology, 100 Suppl.: S125.(also pg. 25 *in* Proc. Plant Pathol. Soc. Alberta, October 12−14, 2010, Lethbridge, AB.).

2009

Ahmed, H.U., Hwang, S.F., Gossen, B.D., Chang, K.F., Howard, R.J., and Turnbull, G.D. 2009. Sensitivity of *Ascochyta rabiei* to pyraclostrobin in western Canada in 2007. Can. J. Plant Pathol: 31: 117.

Bassendowski, K.A., and Gossen, B.D. 2009. Evaluation of penthiopryad fungicide for control of ascochyta blight in chickpea, 2007 and 2008. Report #51 *In* 2008 Pest Manage. Res. Rep. 47: 176–178. Available at www.cps-scp.ca/Pest_Mangement-reports.shtml.

Gossen, B.D., Conner, R.L., and Wolf, T.M. 2009. Spray nozzle configuration affects foliar disease severity on field pea and chickpea. Can. J. Plant Pathol: 31: 485

Gossen, B.D., Hwang, S.F., Thaher, N.H., and McDonald, M.R. 2009. Insensitivity of *Ascochyta rabiei* to strobilurin fungicide in Canada, 2007. Can. J. Plant Pathol: 31: 486.

2008

Ahmed, H.U., Hwang, S.F., Gossen, B.D., Chang, K.F., Howard, R.J., and Burke, D. 2008. Chemical control of ascochyta blight (*Ascochyta rabiei*) of chickpea. Can. J. Plant Pathol: 30: 367.

Ahmed, H.U., Hwang, S.F., Chang, K.F., Gossen, B.D., Howard, R.J., Strelkov, S.E., and Turnbull, G.D. 2008. Influence of repeated infection of a single chickpea cultivar on aggressiveness of *Ascochyta rabiei* isolates. Proc. 7th Can. Pulse Res. Workshop, Winnipeg, MB, Nov. 4–7, 2008. pp. 138–139. Available on-line at www.manitobapulse.ca/pdf/CPRW%20Extended%20Abstracts.pdf

Dokken, F.L., Risula, D., Pearse, P.G., Gan, Y., McDonald, C., Gossen, B.D., Banniza, S., Ta'ran, B., and Goodwin, M. 2008. Chickpea sentinel plant project - developing an early warning system for ascochyta blight. Proc. 7th Can. Pulse Res. Workshop, Winnipeg, MB, Nov. 4–7, 2008. pp. 89–90. Available on-line at www.manitobapulse.ca/pdf/CPRW%20Extended%20Abstracts.pdf

Dokken, F.L., Risula, D., Pearse, P.G., Gan, Y., McDonald, C., Gossen, B.D., Banniza, S., Ta'ran, B., and Goodwin, M. 2008. Update on the chickpea ascochyta sentinel

plant project. Proc. Plant Pathology Soc. Alberta. Oct. 20-22, Lloydminster, AB. p. 8–9.

Gossen, B.D., Chang, K.F., Hwang, S.F., and McDonald, M.R. 2008. Change in sensitivity of *Ascochyta rabiei* to strobilurin fungicides in Saskatchewan, 2002–2006. Can. J. Plant Pathol: 30: 384

Technology Transfer

Barker, B. 2011. *Ascochyta* pathogen insensitive to strobilurin fungicides in chickpeas. Top Crop Manager (West), February 2011, pg. 46–49. Article based on interview with Dr. Gossen.

Bassendowski, K.A., and Gossen, B.D. 2010. Efficacy of serenade ASO Biofungicide on ascochyta blight of chickpea in Saskatchewan, 2010 Final Report to AgraQuest for contract studies in 2010.

Bassendowski, K.A., and Gossen, B.D. 2010. Efficacy of penthiopyrad and picoxystrobin fungicides on ascochyta blight of chickpea in Saskatchewan, 2010. Final Report to DuPont for contract studies on chickpea in 2010.

Bassendowski, K.A., and Gossen, B.D. 2009. Efficacy of LEM17 fungicide on ascochyta blight of chickpea in Saskatchewan, 2009. Final Report to DuPont for contract studies in 2009.

Bassendowski, K.A., and Gossen, B.D. 2009. Efficacy of AGRESS and IOT-SP fungicides on ascochyta blight of chickpea in Saskatchewan in 2009. Final Report to Innovotech for contract studies in 2009.

Gan, Y., Gossen, B.D., Wolf, T., McDonald, C., Poppy, L., Ford, G., Leshures, R., Warkentin, T., Banniza, S., Pearse, P., Dokken, F., and McVicar, R. 2009. An alternative approach to manage ascochyta blight in chickpea. AAFC Bull. No. 10944E. AAFC.

Gossen, B.D. 2009. 2009 Pulse disease watch. FarmStars Insight Newsl. 14 (Spring 2009): 2. (BASF Canada).

Gossen, B.D. and Peters, R.D. 2009. Fungicide insensitivity in field crop pathogens. Invited presentation, Pesticide Resistance Workshop, Saskatchewan Certified Crop Advisors, February 25, 2009. Saskatoon, SK.

Kutcher, H.R., Turkington, T.K., and Gossen, B.D. 2009. Integrating foliar fungicides into your disease control program for maximum benefit. Invited presentation, Crop Production Show, Saskatoon, SK. Jan. 14, 2009.

Thaher, N.H., Gossen, B.D., and McDonald, M.R. 2009. Development of a screening method for detecting fungicide insensitivity to pyraclostrobin. Presentation at the Ontario Pest Management Conference, October 2009, the South Western Ontario section of CPS, London ON October 2009, and the Saskatchewan section of CPS, Saskatoon SK, December 3, 2009.

(c) Personnel

	FTE (per year)	Cost to Project
Professional		
- B.D. Gossen	0.2	-
- M.R. McDonald, U of Guelph	0.2	-
- S.F. Hwang (Alberta Agric., Edmonton)	0.1	-
- K.F. Chang (Alberta Agric., Lacombe)	0.1	-
Technical		
- K. Bassendowski	0.2	-
- Other technical staff	0.1	-
Graduate Student	1.0	$17 K per year

(d) Equipment - none

(e) Photos - A limited number of the pictures used in presentations and posters are available upon request.

(f) Acknowledgements - ADF was acknowledged in all presentations and publications.

(g) Public Information Materials – Presentations based on this work have been made to numerous research and extension professionals, and have been made available to re for use in extension to producers across the region. A scientific paper has been prepared and submitted.

(I) Deliverables for evaluation

Presentations have been made at extension meetings and scientific conferences (see publications section).

Expenditures

A statement of expenditures for the project will be provided under separate cover. It took some time to recruit a suitable graduate student for this project. As a result, salary expenditures were delayed initially to until the student was in place, but the program was completed on time and on-budget.